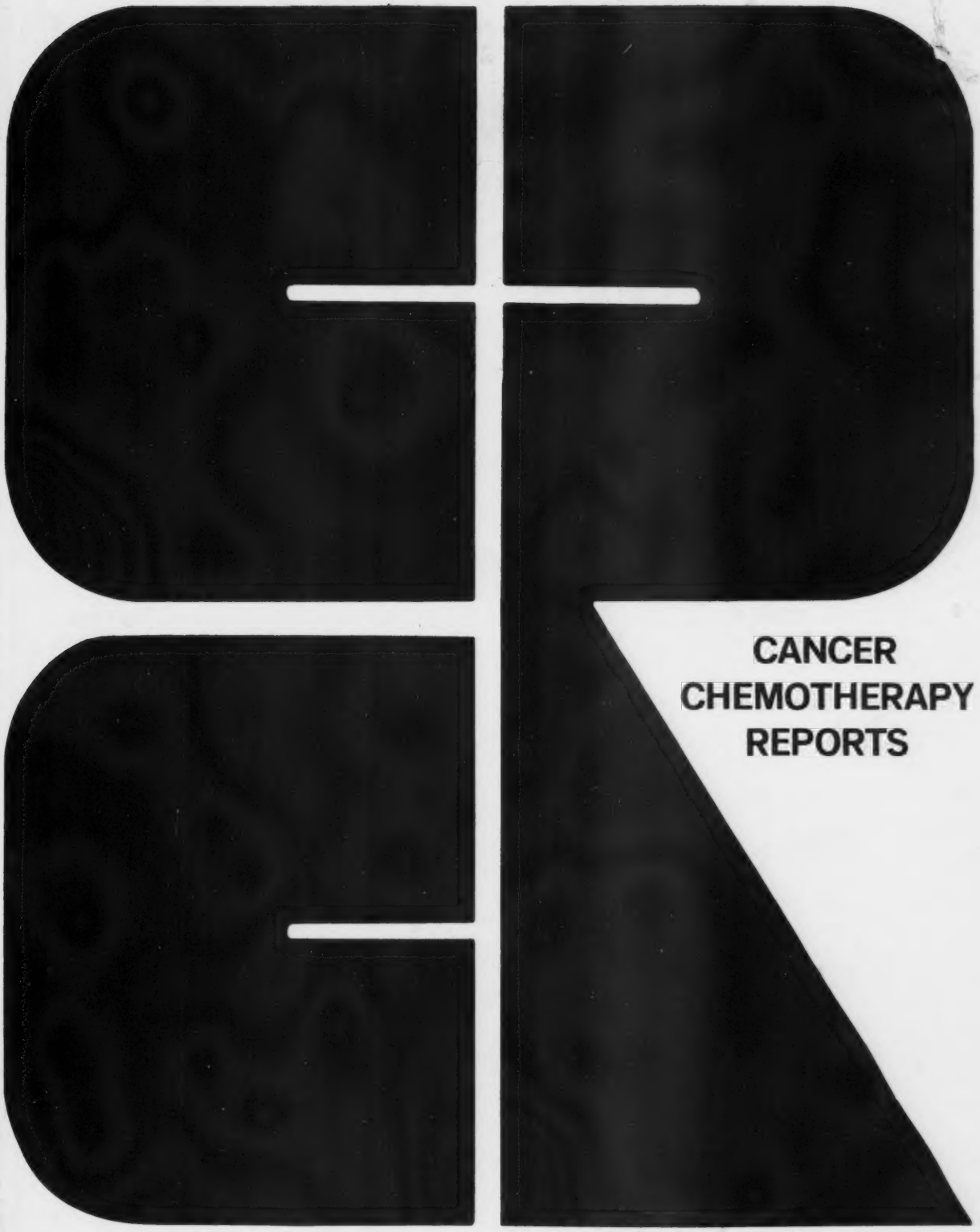

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The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through November 30, 1976.

LETTERS

Potential Hazards Associated With the Use of 1-Methyl-1-Nitrosourea (NSC-23909)

1-Methyl-1-nitrosourea (CAS reg. No. 684-93-5) is one of a series of substituted ureas shown to be active against a variety of animal tumors (1–3). Recently, Emanuel et al (4) have reported that 1-methyl-1-nitrosourea has clinical activity against undifferentiated carcinoma of the lung and Hodgkin's disease; the adverse effects noted by this group were related to the acute toxicity of the drug, eg, nausea, vomiting, and myelodepression. This report by Emanuel will undoubtedly encourage further work with 1-methyl-1-nitrosourea. Therefore, we would like to alert both experimental oncologists and clinicians to some potential serious hazards associated with its use.

Animal studies have shown that 1-methyl-1-nitrosourea is an active mutagen in the dominant lethal assay (5), that it breaks chromosomes in vitro (6), that it is highly teratogenic in the rat (7), and that it possesses potent carcinogenic activity in three strains of mice (8,9), three strains of rats (8,10,11), and the rhesus monkey.[1] In some animal experiments, carcinogenic effects were observed at doses closely approximating the optimal dose in man as reported by Emanuel et al(4).

In addition to these biologic hazards, the chemical properties of the compound also present special difficulties. It is unstable in aqueous solution, with a half-life of 1.2 hours at pH 7 and 0.1 hour at pH 8 (12). Therefore, if suitable precautions are not taken, the decomposition of 1-methyl-1-nitrosourea at room temperature could lead to serious inaccuracies in dosage. Moreover, at room temperature, the drug is sufficiently explosive to blow the lid off and even shatter the container in which it is stored, the compound thus becoming dispersed over a wide area (13,14).[2]

This letter is not intended to discourage the experimental and clinical use of 1-methyl-1-nitrosourea, but rather to caution experimental oncologists and clinicians about the potential biologic and physical hazards that the use of the drug may present.

[1] Adamson RH, unpublished observation.

[2] Rall DP, personal communication, National Institute of Environmental Health Sciences, Research Triangle Park, NC. Dalgard DW, personal communication, Hazleton Laboratories, Vienna, Va.

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Use of Ineffective Agents in Combination Chemotherapy

Although I agree with Bunn (1) that the working hypothesis of combining drugs which have shown effectiveness when used singly has been a useful one, it should not preclude the much needed continued studies of various combinations, including those which use agents relatively ineffective when used alone. The area of combination chemotherapy is still in its infancy and truly tested basic principles are difficult to find for this burgeoning field. Because there are so many variables involved (such as scheduling and dosages) and so many drugs to submit to combination (each of which should be studied in each tumor type), categorical statements will probably remain sparse for many years.

I can cite two papers (there are probably many more) which suggest that a relatively ineffective agent may enhance the effect of a second drug. Kovach et al (2) reported a nil response to BCNU in carcinoma of the pancreas but a 33% response when it was used with 5-fluorouracil, although response to 5-FU alone was 16%.[1] Livingston et al (3) noted that responses with vincristine and bleomycin in combination were superior to those for bleomycin alone.[2]

A related problem is combination therapy using a drug that has been previously administered singly to the same patient. The combination is resorted to because of an initial lack of response or a relapse after an initial response, yet most protocols of combination therapy do not preclude the use of the previously employed drug. The evidence is not conclusive, but response rates appear similar between those who have and those who have not received prior single-agent therapy. Examples of this situation are the use of the Cooper regimen including 5-FU in a patient now refractory to 5-FU or the MOPP program[3] in a patient previously treated with an alkylating agent alone.

Although *cis*-diamminedichloroplatinum[4] may not be very effective in colon cancer, its use in combination therapy can only be assessed by clinical trials. Numerous groups have suggested its use in combination (4–6), mainly because of its mild hematologic toxicity.

It is particularly distressing to find that Kovach et al (7) did not suggest that "the drug *should* be used in trials of combination regimens." They merely stated that their negative results "should not mitigate against" its evaluation in combination.

---

[1] BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

5-Fluorouracil: NSC-19893; CAS reg. No. 51–21–8; 5-FU.

[2] Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

Bleomycin: NSC-125066.

[3] MOPP = mechlorethamine, vincristine (Oncovin), procarbazine, and prednisone.

Mechlorethamine: NSC-762; CAS reg. No. 55–86–7; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride.

Procarbazine: NSC-77213; CAS reg. No. 491–88–3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride.

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-.

[4] *Cis*-diamminedichloroplatinum: NSC-119875; CAS reg. No. 15663-27-1.

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May 23, 1974

In his recent letter, Bunn (1) questions the value of using agents found to be ineffective when used alone in combination therapy, and he proposes that such use is generally unwarranted. As an example he quotes the work of Kovach et al (2), who have recommended trials using the new drug, *cis*-diamminedichloroplatinum,[1] in combination therapy although the drug appears to have little value as a single agent against colorectal carcinoma. This appears to be an injudicious example, since recent results, which have not yet appeared in print, have proved the merit of the recommendation of Kovach et al. I will briefly review the relevant results.

This platinum drug is the first inorganic complex to be entered into extensive clinical trials by the National Cancer Institute. Since it represents a drastic departure from the usual classes of cancer chemotherapy agents it should be most carefully evaluated, as it may be the paterfamilias of a large group of chemicals that have not been adequately surveyed for antitumor activity. This drug has been extensively evaluated against a broad spectrum of animal tumor systems before entering clinical trials. These results showed it to be one of the most potent, broad-spectrum antitumor agents yet developed (3). It is now the positive control in the National Cancer Institute screening tests against B16 melanoma.

Phase I clinical trials have established a number of acceptable dose levels under different schedules, and phase II clinical trials are under way at present. The drug already appears to be an effective agent against tumors of the genitourinary tract and objective remissions have sporadically been observed in a wide variety of other tumor types. Higby et al (4) have shown responses in 13 of 15 testicular tumor patients, seven of which were complete and of long duration. Wiltshaw (5, and personal communication) has treated 25 terminal ovarian carcinoma patients, obtaining ten objective responses, four of which are complete and of long duration. Thus, in at least two tumor classes where a sufficient number of cases have been evaluated, good response rates have been obtained using the drug as a single agent. The Kovach et al study is the third with sufficient patients entered to allow an evaluation, and their evidence is that the single drug is of little value against colon carcinoma.

Woodman et al (6), Schabel (Southern Research Institute Report), and Speer et al (7) have shown that in animal tumor systems (L1210 leukemia) the platinum drug is synergistic, or at least additive, in combination therapy with a wide variety of established antitumor agents. These results form the basis

[1] *Cis*-diamminedichloroplatinum: NSC-119875; CAS reg. No. 15663-27-1. It will be noted in the references to this Letter that no two authors name this drug alike. According to IUPAC conventions, the definitive name is *cis*-dichlorodiammineplatinum (II).

for the consideration of using the platinum drug in combination therapy, and thus the suggestion by Kovach et al for its use in such therapy despite the limited results when tested against colon carcinoma as a single agent.

Their suggestion has now been substantiated. Ellerby et al (8) have undertaken phase I clinical trials of the platinum drug combined with 5-fluorouracil.[2] Their first report includes nine terminal colon carcinoma patients. Objective responses were observed in three of these patients as follows: (*a*) colon adenocarcinoma and chronic lymphocytic leukemia, 50% decrease in nodes and no measurable lesions of 8 months' duration and continuing; (*b*) colon adenocarcinoma, 50% decrease in abdominal mass of 3 months' duration; (*c*) colon adenocarcinoma, 50% decrease in tumor and liver metastases of 4 months' duration and continuing. This last patient had already failed to respond to multiple daily doses of 5-FU and intrahepatic arterial perfusion of 5-FU and yet he responded to combination therapy with conventional 5-FU plus the platinum drug.

While it would simplify chemotherapy testing to have such a general rule as suggested by Bunn, that is, "when an agent is ineffective alone, its use in combination trials is generally unwarranted," I have described above a clear-cut case that invalidates such a rule. I suggest, instead, that if any evidence from animal trials can lead to an inference of synergism, or even additivity, for a specific combination therapy, then this therapy should be attempted in clinical trials, even if either or both of the individual drugs are separately ineffective against the given tumor type. We are still too much lacking in effective therapies for colon carcinomas to close off potentially valuable drugs and too much lacking in effective theories of chemotherapy to allow general rules to supercede experimental tests.

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June 21, 1974

The preceding letters of Drs. Tashima and Rosenberg provide references which they claim support the principle that agents which are ineffective alone can be additive or synergistic when combined with effective agents. In fact, none of the papers cited demonstrate such enhancement although they do highlight many difficulties encountered in interpreting clinical studies. Additive or synergistic responses can only be proven when the effect of each single agent for a given tumor is known and when the combination of drugs is statistically superior to each alone, especially when given in a random, controlled manner. This significant difference should affect both the response and survival rates. The cited reports of Kovach et al (1), Livingston et al (2), and Ellerby et al (3) do not provide adequate proof of such an additive or synergistic effect.

[2] 5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU.

The principle of testing combinations of single agents which are effective against a given tumor, especially when synergistic in animal tumor systems, is not disputed. Numerous examples of the value of such combinations have been demonstrated in human tumors (4,5). I am still unaware of reports showing a beneficial effect of a drug used in combination when it is ineffective alone. *Cis*-diamminedichloroplatinum [1] is effective *alone* and has been shown to enhance the response to other effective agents in certain animal systems (6). It may be effective alone in some human tumors (7), and if so I would not question the value of testing combinations in such tumors. However, the absence of effect, except toxicity, when used alone in colon cancer (8) should mitigate against combination testing in this tumor. Can we afford to test a myriad of ineffective agents in combination when we have so much to learn about agents which are effective alone and combinations of effective agents?

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July 10, 1974

The simultaneous publication of Dr. Bunn's letter (1) concerning ineffective agents in combination chemotherapy and the article entitled "Treatment of Advanced Gastrointestinal Cancer with Emetine (NSC-33669)" (2) prompts me to offer an affirmative answer to the question of such use of ineffective single agents.

Admittedly, the study by Moertel et al (2) was concerned with emetine (CAS reg. No. 316-42-7) as a single agent for carcinoma of the gastrointestinal tract and I am presently engaged in a clinical trial using emetine in combination therapy for lung cancer. Even so, the final sentence of the Discussion, coming as it does from the prestigious Mayo Clinic, might be taken by many as the death knell for emetine as a weapon in the fight against cancer.

Although a relative tyro in the field of cancer chemotherapy, I have involuntarily made this sideways step from the chemotherapy of tuberculosis. In this discipline we learned that drug combinations were more effective than single agents, and that a drug did not need to be particularly potent against the tubercle bacillus to have an important place in antibacterial chemotherapy. For 20 years sodium paraamino salicylate (PAS) was rated one of the three first-line drugs in the chemotherapy for tuberculosis, owing its position mainly to its ability to prevent the development of organisms resistant to the other two more potent agents. Used alone in the chemotherapy of tuberculosis, PAS could fairly be described as an ineffective agent.

[1] *Cis*-diamminedichloroplatinum: NSC-119875; CAS reg. No. 15663-27-1.

This letter is not intended as a criticism of the excellent work of Moertel et al in assessing the value of emetine in gastrointestinal cancer, but I must point out that only two dose regimens were tested, and, even as a single agent, there could be better results if emetine were used differently. In a paper (3) presented by Professor Lucien Israel of Paris, France, earlier this year, promising results were reported by giving weekly injections of dehydroemetine [1] in a dose of 3 mg/kg to 50 patients with disseminated carcinoma which had proved unresponsive to other drugs. Among these were a few patients with gastrointestinal cancer.

I have been treating advanced lung cancer with a combination of emetine and cyclophosphamide [2] for more than 2 years and in all cases emetine has been given by weekly intravenous injections. Having found both drugs to be of very limited value when given alone, I was immediately impressed by the remarkable synergism that they showed in combination. The deciding factor in my choice of this combination was the completely different toxicities of these two drugs. It would be difficult to present a case for using a totally ineffective agent in combination chemotherapy, but a drug of limited cytostatic potency may well have an important role in cancer chemotherapy provided its toxicity is not additive to the more potent agents with which it is combined.

[1] Dehydroemetine: NSC-129414; emetine, 2,3-didehydro-, dihydrochloride, (±)—.

[2] Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate.

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GUEST EDITORIAL

Historical Controls Reincarnated: An Examination of Techniques for Clinical Therapeutic Research

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The technique of the randomized comparative clinical trial has gained wide acceptance among clinical investigators. There is little basis for objecting to this since a large body of useful clinical information has been derived utilizing this technique. However, there are several symptoms within the biomedical community which suggest that the acceptance of this clinical trial methodology has resulted in the interdiction of investigation for new, potentially better techniques. Perhaps more important, a new anti-intellectualism has arisen based on the notion that the perfect technology has been achieved, thus any variation from the standard format represents a deviation from "true science." Many individuals, particularly in agencies of the government, have come to regard clinical research which does not utilize the standardized format as "unscientific." As a result, the search for novel ways of conducting clinical research is being deterred and, perhaps more important, the limitations in techniques for conducting clinical trials are being ignored. In this editorial, some of these limitations will be discussed in order to encourage continued progress in therapeutic research.

Six aspects of the present widely practiced "controlled" clinical trial will be considered. These are: (*a*) research objectives; (*b*) the allocation of patients at random; (*c*) the use of placebo or other types of blinding mechanisms; (*d*) the simultaneous study of two or more regimens, that is, concurrent evaluation; (*e*) the two-sided test, ie, better or worse than the standard treatment; and (*f*) the use of prospective data in contrast to historical data.

Research Objectives

The essential ingredient of clinical research is a clinically significant objective. One cannot replace the intelligent, imaginative, creative work of a clinical scientist and scholar by the routine application of the clinical trial technique. A sophisticated clinical trial which gives highly statistically significant results may, of course, be of no clinical value to any patient. In contrast, the imaginative application of original clinical research can result in dramatic alterations in medical practice. Examples of this are too numerous to cite, but the discovery of mechlorethamine, of the first antimetabolite methotrexate, of the drug vincristine, of the antitumor effect of prednisone, of multiple drug combination chemotherapy, etc, were all dramatic advances in the outlook for patients with malignant disease. For these discoveries clinical investigators thoroughly familiar with both the diseases under study and the effects of treatment devised novel approaches and observed results which were substantially better than for any previous therapy. Clearly, the best clinical trials are still the ones that have the best treatments in them.

Randomization

The major purpose of random allocation of patients is to assure equal distribution of *unknown* variables which might affect the results. The intellectual basis for random allocation is secure. However, when each patient characteristic is considered, nearly equal proportions of patients will be on each treatment only when the number of patients is large. If the number of patients is small or moderate, particularly when the number of variables which

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affect the result is large relative to the number of patients, the probability of an unequal proportion of patients on each treatment for some of the patient variables is quite high. The defect in randomization is most glaring when variables known to affect response are not used for stratification and randomization alone is used. An example is in acute leukemia where age has a profound effect on the probability of response. If one is to study the effect of a treatment for acute leukemia, it would be important to correct for the age variable when comparing treatments between groups. Random allocation of patients without regard to the age variable would frequently result in an unequal distribution of patients and results could be biased. Other examples include the known effect of the extent of disease or the so-called "staging" of malignant disease, and the presence of complications which are known to diminish the prospects of response such as infection, bone marrow failure, prior therapy, etc. It is important that when there are a reasonably large number of variables known to affect response, comparison of different treatments should be within patient groups that have comparable pretreatment characteristics. This can be accomplished by stratification and/or the matching of individual patients. In such clinical studies, the number of stratifications required results in a small number of individuals in each group and then randomization within stratifications of patients is less important than the choice of prognostic categories.

Placebo or Blind Studies

Many cancer studies utilize drugs in a coded fashion which conceals the identity of the drug from the patient and physician. This "double-blind" technique was introduced into clinical trials when the subjective component of the evaluation was important. It provides protection against either patient bias (ie, the placebo-response effect) or investigator bias. However, a much preferred technology is the use of objective, quantitative criteria for defining treatment and response. Examples would be the use of objective measurement of either tumor size or extent of disease; in leukemia, the measurement of white blood cell and platelet counts by an instrument; the requirement for changes of drug therapy based on objective findings, ie, the escalation of dosage based upon objective changes in peripheral blood values or the discontinuation of drugs due to toxic side effects such as decreased knee reflex, decreased hematologic values, or stomatitis. The serious disadvantage of blinding the drug is the interference with the normal professional relationship between a physician and his patient. For clinical research to progress satisfactorily the essential ingredient is "informed consent." It is important that both physician and patient have sufficient information about the medication being administered to allow an intelligent judgement as to whether they wish to participate. Techniques which interfere with the patient-physician relationship are, of course, detrimental and should not be resorted to except in circumstances when treatments can only be evaluated by subjective criteria.

Concurrent Studies

The concept that disease is changing progressively with time is based on a few dramatic examples such as influenza. However, there are a number of malignant diseases in which the pattern of spread of illness has been quite regular and reproducible for many decades. In diseases such as leukemia, cancer of the lung, colon, breast, and others, these patterns are quite predictable and much of the variation from patient to patient can be accounted for by pretreatment variables known to affect response. Therefore, in the absence of any evidence that the disease has changed over a period of time and in the presence of a large body of historical knowledge, the concept of investigating a small number of patients prospectively to determine whether there has been a change in the disease process is illogical and interferes with clinical research. Observations made in any small group of patients can give only a limited degree of precision of the knowledge of the natural history of a disease when compared to observations made in many thousands of patients in many different centers throughout the world. It is the historical data which has the burden of statistical probability based both on the reproducibility of the measurements made over and over again and

the size of the sample being investigated by objective criteria. Moreover, concurrent comparison has a major disadvantage. If one is simultaneously investigating both a new treatment and a control treatment, the probability of obtaining a result which differs from the "true value" is just as great in both groups. There are many examples in the literature where the so-called control data differs significantly from the historical data which is not only larger in quantity but has been consistently reproduced over many years. In such a circumstance, one might be led to believe that a new treatment is either better or worse than the established treatment based on a poor measurement in the control series. Similarly, the risk of observing a falsely high response rate on the new treatment (which is not confirmed in subsequent studies) is not diminished by having a concurrent control group. In fact, the division of the study patients into two groups leads to a less precise evaluation of a new treatment compared with assigning all patients to the new treatment. The quality of evaluation of a new treatment depends upon having treated a group of patients which is representative of the total population and fairly large so that confidence intervals for response rate will be narrow. Therefore, having a concurrent control group is generally not an important advantage and should be used only in circumstances where there is clear evidence of a change in the natural history of disease over a period of time.

The Two-Sided Test

In clinical investigation, we seek results which differ in that the overall prognosis for the patient is better. Clinical trials in which the patients do less well than they have in the past are to be avoided at all cost and to be concluded as early as possible. Clinical trials designed to find a treatment which is better than a control treatment test a "one-sided" hypothesis of whether the new therapy is *better* than the standard or not, rather than a "two-sided" test of whether or not there is a difference in either direction from the standard. A comparative clinical trial should not be started unless the preliminary data suggest that the new therapy is at least as good and possibly better than the standard. If this is accepted, the question can be raised whether it is ethical to enter patients on the standard therapy when there is little or no chance that the standard therapy could be better than the new therapy. It would be small consolation indeed for patients on the standard therapy to learn that they have been entered for "control purposes." Having a philosophy of always looking for better treatments makes it difficult to test the neutral "two-sided" hypothesis. Thus, the useful approach of using pilot studies of small series of patients, making objective measurements, and confirming by larger clinical trial only the potentially positive results has in the past and will in the future be an important technique for clinical research.

Prospective Data

The concept of a prospective clinical trial is perhaps the most misleading of all of the principles of a clinical trial. There is, of course, no prospective knowledge. Once a "prospective" trial is completed, the result of that trial becomes "historical" information, that is, its truth or lack of truth is dependent entirely on whether that result will predict for the future. The fundamental basis for the scientific accumulation of information is the observation of events as they occur in a systematic way, using objective measurements if possible. These observations are then analyzed and formulated in a statement which predicts for the future, such as, "administration of drug A in manner B will result in frequency of objective response C with a duration of D." That prediction will prove useful if the result is confirmed in subsequent studies of larger numbers of patients. Repeated confirmation of results observed in the past makes the historical fact a more useful addition to our fund of knowledge. The concept that the historical control is suspect is based upon choice of inappropriate or poorly selected historical controls. There is, of course, an element of anti-intellectualism in the prospective control concept which suggests that the historical facts not only may be, but *must* be ignored. Thus, the suspicion that dramatic changes may occur in the natural history of

the disease or that unexpected events may occur in the future which have not been observed in the past has been used to justify ignoring observations made in thousands of patients treated in the past, and conducting comparative, prospective clinical trials in all circumstances.

SUMMARY

The prospective, double-blind, randomized, concurrent clinical trial is not the *only* technique useful for evaluating therapy. In fact, it is generally not the best way to do such research. The identification of prognostic variables which allows the comparison between the group under investigation and other groups of patients investigated in the past provides a powerful technique for evaluating new treatment. Recording observations which differ significantly from those made in the past forms the basis for new knowledge. Confirmation of these observations by the same investigator and by other investigators in other institutions provides an effective mechanism for generating knowledge which predicts for the future. Progress in medicine and in other human affairs has resulted in the past from expanding the horizons of the processes of human intellectual activity. The creation of artificial barriers, ie, the proscription of the consideration and utilization of new techniques, has always had an anti-intellectual and impeding function which restricts the rate of accumulation of knowledge beneficial to humanity. There is no substitute for imaginative, original, and creative thought. The use of good techniques is not sufficient. The careful analysis of past results, as a basis for the formulation of significant hypotheses, followed by objective, scientific, and valid evaluation has served mankind well in the past and almost certainly will in the future. The latter confidence is based on "historical controls," hopefully to be fully *"reincarnated."*

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Effect of Combined Surgery and BCNU (NSC-409962) on an Animal Brain Tumor Model [1,2,3]

Esref Tel, [4,5] Marvin Barker, [4] Victor A. Levin, [4,6] and Charles B. Wilson [4,7,8]

SUMMARY

Rats bearing intracerebral tumors were used to determine the effect of combined surgery and BCNU therapy. BCNU administered on Day 16 after tumor implantation resulted in a more significant increase in lifespan than treatment with BCNU on Day 10. Surgery performed more than 3 days before or after treatment with BCNU was less effective than BCNU alone.

[Cancer Chemother Rep 58:627–631, 1974]

Experimental chemotherapy of brain tumor models in mice and rats has had moderate influence on the direction of clinical chemotherapy, but certainly the value of these models has not approached the importance of the L1210 system to therapeutic advances in acute leukemia of childhood. The murine ependymoblastoma screen established by Shapiro, Ausman, and Rall identified the nitrosoureas as effective agents in contrast to mithramycin, 5-fluorouracil, vincristine, and cyclophosphamide which were shown to be inactive (1,2). In our laboratory, the 1-methyl-1-nitrosourea-induced rat gliosarcoma has been used extensively to study cell kinetics, immunology, and aspects of experimental chemotherapy. The intracerebral rat sarcoma has been shown to have a low growth fraction (10%–20%) and to be sensitive to BCNU when the tumor reaches a symptomatic size (3–6).

In an effort to approximate more closely the clinical situation of chemotherapy following subtotal tumor removal and to investigate the relative benefits of BCNU given before and after surgery, we undertook the following experiments in rats bearing cell culture-derived intracerebral tumors.

METHODS AND MATERIALS

Animal Model

The tumor used in this study was induced in Fisher rats with 1-methyl-1-nitrosourea (7). Forty-eight-hour cultures of this tumor

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[2] Supported by Public Health Service grant CA-13525 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare; and gifts from the Phi Beta Psi Sorority and the Joe Gheen Medical Foundation.

[3] BCNU was supplied by Drug Research and Development, Division of Cancer Treatment, NCI. Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

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[5] Participant of the Department of State Agency for International Development (AID), PIO/P No. 277-2600314.

[6] Departments of Neurology (School of Medicine) and Pharmaceutical Chemistry (School of Pharmacy), University of California, San Francisco.

[7] We wish to acknowledge that the tumor used in this study was originally provided by William H. Sweet, Paul T. Kornblith, Janette R. Messer, and Beverly O. Whitman, Massachusetts General Hospital, Boston. The technical assistance of Mary Freeman, Kathy Knebel, and Maria Misiaszek, and the histological assistance of Dr. Surl Nielsen are greatly appreciated.

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were harvested by trypsinization (0.25%) for 15 minutes and adjusted to a concentration of 4×10^6 cells/ml in Gev's balanced salt solution (3,4). Adult male Fischer 344 rats were anesthetized with pentobarbital and alcohol and placed in a small animal stereotaxic head holder. The scalp was opened electrosurgically and a 0.07-inch hole was drilled at a point 2.5 mm to the left of the midline and 4.0 mm anterior to the frontal zero plane according to the atlas of Konig and Klippel (8). The hole was fitted with a stainless-steel 0.125 × 0.0625-inch screw with a 0.0292-inch center-drilled hole. A blunt 22-gauge needle attached to a 0.1-ml chromatography syringe (Hamilton) with the needle extending 3.0 mm beyond the screw was used to inject 0.01 ml of the cell suspension into the centrum ovale. The central screw hole was immediately sealed with bone wax to prevent reflux and the scalp incision was closed with a skin clip. Fewer than 1% of the animals died from this procedure.

Surgical Procedure

After craniotomy, the tumor was grossly removed using microsurgical techniques. Excised tumor tissue was weighed and representative pieces were submitted for histologic examination.

Fewer than 1% of the animals undergoing operation died within 5 days of surgery. For statistical purposes, however, only animals surviving 5 days after surgery were included in the data presented in tables 1 and 2.

BCNU Administration

BCNU was obtained in vials containing 100 mg of BCNU and 400 mg of mannitol. For reconstitution, 3 ml of absolute ethanol and 27 ml of sterile water were added. Immediately after reconstitution, 1.0 ml of the drug solution was administered by intraperitoneal (ip) injection at a dose equivalent to 16.7 mg/kg. This dose was 1.2 of the reported LD10 value (9) and represented the highest nontoxic tolerated dose.

Therapy Schedules

Tumor-bearing rats were randomly divided into the following groups:

A. Control or untreated

TABLE 1.—%ILS (treated median survival × 100/control median survival) and the significance (*P*) of each experimental group, as analyzed by a Wilcoxon rank sum analysis

	Experiments*								
	1			2			3		
Groups	No. of animals	%ILS	*P*	No. of animals	%ILS	*P*	No. of animals	%ILS	*P*
Control	10	—	—	10	—	—	10	—	—
BCNU (Day 10)	10	160	<0.0001	10	160	<0.0001	10	162	<0.0001
BCNU (Day 16)	10	222	<0.0001	10	213	<0.0001	10	200	<0.0001
BCNU (Day 10) + Surgery (Day 13)	10	186	<0.0001	10	182	<0.0001	10	183	<0.0001
BCNU (Day 10) + Surgery (Day 16)	10	200	<0.0001	10	180	<0.0001	10	175	<0.0001
Surgery (Day 13) + BCNU (Day 16)	10	172	<0.0001	10	127	0.002	10	170	<0.0001
Surgery (Day 13)	14	128	<0.0001						

*Median for the three groups was 23 ± 2 (SE) days.

TABLE 2.—Relative increase in lifespan (RILS) (median A survival × 100/median B survival) and significance (*P*) of their differences summarized for all drug and surgery combinations in the three separate experiments

A \ B	EXPERIMENT NO.	BCNU (day 10)		BCNU (day 16)		BCNU (day 10) SURGERY (day 13)		BCNU (day 10) SURGERY (day16)		SURGERY (day 13) BCNU (day 16)	
		% RILS	P	% RILS	P	% RILS	P	% RILS	P	% RILS	P
BCNU (day 10)	1			70	<.00001	85	.002	79	<.00001	92	NS
	2			74	.007	86	NS	88	NS	120	.02
	3			82	<.00001	89	.01	94	.01	96	NS
BCNU (day 16)	1	140	<.00001			119	<.00001	110	<.00001	128	<.00001
	2	134	.0007			117	<.00001	119	.00004	160	.009
	3	124	<.00001			103	.0001	114	.013	117	<.00001
BCNU (day 10) SURGERY (day 13)	1	117	.002	83	<.00001			96	.003	107	.006
	2	115	NS	85	<.00001			101	NS	140	NS
	3	110	.017	90	.0001			104	NS	107	NS
BCNU (day 10) SURGERY (day 16)	1	125	<.00001	83	<.00001	107	.003			116	<.0001
	2	113	NS	83	.00004	97	NS			140	NS
	3	106	.01	86	.013	90	NS			102	NS
SURGERY (day 13) BCNU (day 16)	1	108	NS	70	<.00001	92	.006	82	<.00001		
	2	80	.02	59	.009	70	NS	71	NS		
	3	104	NS	85	<.00001	93	NS	90	NS		

B. BCNU given on Day 10
C. BCNU given on Day 16
D. BCNU given on Day 10 and surgery performed on Day 13
E. BCNU given on Day 10 and surgery performed on Day 16
F. Surgery performed on Day 13 and BCNU given on Day 16
G. Surgery performed on Day 13

This protocol was repeated three times in experimental groups A–F (each containing ten animals) and once in group G (14 animals).

Determination of Therapeutic Effect

All animals were observed daily and the day of death was recorded. The results were analyzed by: (*a*) median survival; (*b*) range of survival; (*c*) increased lifespan (%ILS = median survival of treated animals × 100/median survival of control animals); and (*d*) statistical analysis by the Wilcoxon rank sum test (10). After death, tumors were removed from random animals in all groups and submitted for routine histologic examination.

Tumors taken from animals on Day 13 weighed between 70 and 80 mg. The average weight of surgically removed tumors was 66.6 mg. It was therefore concluded that the portion of tumor remaining, including those cells infiltrating adjacent brain, was approximately 10%.

RESULTS

Median survival of control groups was 23 ± 2 (SE) days. The results of the three experiments are shown in table 1. All treated groups, with one exception, had a highly significant %ILS when compared to the untreated groups ($P < 0.0001$). In experiment 2, the surgery (Day 13) and BCNU (Day 16) group had a %ILS of only 127% ($P = 0.002$). The most prolonged survival was achieved by BCNU given alone on Day 16. BCNU given alone earlier (Day 10) was the least effective form of treatment.

Table 2 compares the intergroup relationship of the various therapeutic combinations. From this table it is apparent that BCNU alone on Day 16 was more effective than BCNU on Days 10 or 13 or when combined with surgery either before or after BCNU. BCNU on Day 10 followed by surgery on Day 13 was more effective than BCNU alone on Day 10 in two of the three experiments; the results of BCNU on Day 10 and surgery on Day 16, however, were better than BCNU on Day 10 in one of three experiments and better than BCNU (Day 10) and surgery (Day 13) in one of three experiments.

Microscopically, all surgically removed tumors and specimens selected randomly from all other groups were found to be neoplastic and of similar histologic type.

DISCUSSION

The purpose of these studies was to develop an animal model which might duplicate the clinical situation common to current phase III studies, namely, incomplete surgical removal followed by chemotherapy (11–13). In the surgery of malignant gliomas it is rare, except possibly in the case of lobectomy or hemispherectomy, to remove more than 90% (eg, 1-log cell loss) of the tumor. Precisely for that reason, our studies involved subtotal removal of 90% of the rat tumor. Under these circumstances, a median survival increase of 28% is reasonable and appropriate for a 1-log cell loss (kill) from surgery based on the cell kinetics of this tumor (3).

Therapy with BCNU alone given 10 days after implantation confirmed results obtained previously (3). We were surprised to find however, that BCNU (Day 16) produced a greater %ILS than BCNU given earlier. The cell-cycle kinetics of this tumor on Day 16 are such that the tumor is considerably larger with a reduced proliferating pool in comparison to the tumor on Day 10 (3,6). It thus appears that BCNU, a cell-cycle nonspecific agent, has a greater antitumor effect against nonproliferating cells. This observation substantiates previous investigations in other systems that demonstrated enhanced chemotherapeutic effectiveness of the nitrosoureas on cells in lag or plateau phases of growth (14,15).[9]

Combination therapy with BCNU before or after surgery was not superior to therapy with BCNU given alone on Day 16 after implantation. This is perhaps not too surprising since surgery alone results in only a 1-log cell kill and BCNU therapy results in at least a 3-log cell kill (16). The improvement in %ILS for BCNU given after surgery compared to surgery alone is quite similar to that reported by Walker and Gehan for the Brain Tumor Study Group phase III study of surgery alone and surgery followed by BCNU (13).

The observation that BCNU given prior to surgery on Days 13 or 16 was slightly better than BCNU given alone on Day 10 could be related to more efficient removal (eg, surgery) of dead or dying cells resulting from the previous BCNU therapy. Further studies are underway to expand these observations to determine the optimal timing for combined BCNU and surgery and to evaluate the tumor growth fraction following surgical pertubation in order to identify the most efficacious time for BCNU therapy.

Probably the most interesting finding of this study was that of the various combinations of BCNU and surgery, BCNU alone on Day 16 was the most effective in increasing lifespan. Along this same line, our clinical experience with deep thalamic gliomas not treated with surgery indicates favorable results with chemotherapy alone in comparison to malignant gliomas treated with surgery followed by chemotherapy (17,18). Confirmation and extension of these laboratory findings

[9] Levin VA, et al. Unpublished data, Sept 1973.

will be necessary before chemotherapy without, or prior to, surgery can be advocated for clinical application.

LIST OF COMPOUNDS

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8

1-Methyl-1-nitrosourea: NSC-23909; CAS reg. No. 684-93-5

Mithramycin: NSC-24559; CAS reg. No. 18378-89-7

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Effect of *Cis*-diamminedichloroplatinum (NSC-119875) on the Allograft Reaction in Mice[1,2,3]

G. Brambilla, M. Cavanna, and A. Maura[4,5]

SUMMARY

***Cis*-diamminedichloroplatinum, administered intraperitoneally in a single dose of 8 mg/kg, significantly depressed skin allograft reaction in mice. Maximum prolongation of graft survival time over control survival time occurred when the compound was injected 24 hours before grafting. Increases in such survival were 39% in mice bearing H-2-incompatible grafts and 238% in mice bearing H-Y-incompatible grafts.**

[Cancer Chemother Rep 58:633–636, 1974]

Cis-diamminedichloroplatinum, a cytotoxic compound from a series of platinum diamines, has been shown to exert antineoplastic effects in animal tumor systems (1–6) and to depress the following immune responses: phytohemagglutinin-induced blastogenesis in human lymphocytes (7), IgM-hemolysin-producing cell formation (8), graft-versus-host reaction (9), and allograft reaction across H-2-histocompatibility barrier (10) in mice. Phase I clinical trials of this platinum salt have been undertaken in patients with advanced neoplastic diseases (11–13). Studies on the mechanism of action showed that *cis*-diamminedichloroplatinum is a potent, selective, and probably irreversible inhibitor of DNA synthesis (2,14).

This study contributes to the knowledge of the immunodepressive activity of this agent on allograft reactions elicited by histocompatibility barriers of different strengths.

MATERIALS AND METHODS

Mice of the C57BL/6Gen and BALB/c inbred strains were used; they were originally obtained as C57BL/6N and BALB/cAnN from the National Institutes of Health, Bethesda, Maryland, and were maintained by continuous brother-to-sister mating by the single line system. Reciprocal syngeneic skin grafts between randomly selected mice of the same sex showed indefinite survival.

Cis-diamminedichloroplatinum was dissolved just prior to use in isotonic saline at a concentration of 0.4 mg/ml and was administered intraperitoneally (ip) in a single dose of 8 mg/kg.

Such a dosage has been found to produce maximum antineoplastic and immunodepressive effects in various systems and to be more effective than divided dosage (1,9,10). The time of administration and the number of mice in each experiment are indicated in the tables.

In order to test the immunodepressive effect of this platinum salt on skin allograft survival across histocompatibility barriers of differ-

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[2] Supported by a grant from the Consiglio Nazionale delle Ricerche.

[3] *Cis*-diamminedichloroplatinum: CAS reg. No. 15663-27-1. Supplied by Drug Research and Development, Division of Cancer Treatment, National Cancer Institute.

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ent strengths, two histoincompatibility models were used. In the first model, C57BL/6 (H-2^b) mice were used as donors and BALB/c (H-2^d) mice were used as recipients; these strains differ at the strong H-2 locus and at a relatively large number of weaker H loci. In the second model, C57BL/6 males were used as donors and C57BL/6 females were used as recipients; males and females of this strain differ at the weak H-Y locus. Transplants were performed according to the technique of Billingham and Medawar (15) in 3–4-month-old mice by grafting approximately 60 mm^2 of ear skin of the donor onto the back of the recipient; criteria adopted to calculate graft survival time and statistics used were previously described (16).

RESULTS AND DISCUSSION

The activity of *cis*-diamminedichloroplatinum in prolonging the survival time of ear skin graft in H-2-incompatible mice is shown in table 1.

To elucidate the relationship between the time of drug treatment and the effectiveness of immunodepressive activity, a single dose of the agent was administered at various times (from Day −3 to Day+4) before and after grafting (Day 0) in eight groups of mice. A statistically significant increase in graft survival time always occurred except when treatment was performed on Day +2. A maximal prolongation of 39.4% over controls was seen in mice receiving the compound on the day before transplantation; such a prolongation is significantly greater ($P<0.05$) than that observed in mice treated on Days −3, −2, +1, +2, and +3.

In mice bearing H-Y-incompatible grafts (table 2) the same dose of *cis*-diamminedichloroplatinum was tested only on Days −1 and +1 in relation to grafting. Prolongation of graft survival time was remarkably greater than in the H-2-incompatibility system and significantly longer in mice receiving the platinum salt the day before the grafting compared to those treated on Day +1.

Our results show that at a dose of 8 mg/kg (the most effective dose in inhibiting tumor growth in mice [1]), the compound exerts a significant depressive activity against immune reactions elicited by transplantation antigens. The prolongation of H-2-incompatible graft survival time was shorter in our experiments than in the experiments of Khan et al (127% over controls) (10). Such a difference can be explained on the basis of (*a*) the histoincompatibility system (characterized by a single H-

TABLE 1.—Survival time of H-2-incompatible skin allografts in mice treated ip with 8 mg/kg of *cis*-diamminedichloroplatinum

No. of mice	Day of treatment	Skin allograft survival time (days) ST50*	19/20 Confidence limits	S†	Increase of ST50 over controls (%)
40	Controls	9.77	9.56– 9.99	1.047	—
8	−3	11.28	10.64–11.97	1.053	15.5‡
8	−2	10.94	10.44–11.48	1.046	12.0‡
7	−1	13.62	12.23–15.18	1.010	39.4‡
6	0	11.32	10.54–12.14	1.059	15.9‡
10	+1	11.41	10.98–11.86	1.042	16.8‡
11	+2	9.91	9.21–10.66	1.077	1.4
7	+3	11.34	10.48–12.28	1.073	16.1‡
7	+4	11.80	10.83–12.86	1.081	20.8‡

* ST50 = time at which the endpoint of graft survival time (complete destruction of the graft) occurs in 50% of the test animals.

† S = slope function (according to Litchfield [17]).

‡ The time distribution of rejections is statistically different from controls at the 5% level according to the Wilcoxon two-sample test (18).

TABLE 2.—Survival time of H-Y-incompatible skin allografts in mice treated ip with 8 mg/kg of *cis*-diamminedichloroplatinum

No. of mice	Day of treatment	Skin allograft survival time (days)			Increase of ST50 over controls (%)
		ST50*	19/20 Confidence limits	S†	
19	Controls	19.96	18.87–21.13	1.085	—‡
7	−1	67.42	52.40–86.74	1.226	238‡
7	+1	39.06	30.42–50.17	1.224	96‡

* ST50 = time at which the endpoint of graft survival time (reduction of the graft to 50% of its original size) occurs in 50% of the test animals.

† S = slope function (according to Litchfield [17]).

‡ The time distributions of rejections are statistically different in any two of the groups at the 5% level according to the Wilcoxon two-sample test (18).

2-histocompatibility barrier), (*b*) the different kind of transplanted tissue (tail skin), and (*c*) the different computation of survival time (mean instead of median) used by Khan et al (10).

In both of our transplantation systems, the maximum immunodepressive effect occurred in groups receiving the compound on Day −1, while Khan observed the maximum effect in mice treated on the day of grafting.

Because of its significant immunodepressive effects against responses elicited by weak antigens, we believe that *cis*-diamminedichloroplatinum could effectively interfere with a possible response of the host against tumor-specific transplantation antigens.

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Comparison of the Acute Hemolytic and Cardiovascular Actions of Ellipticine (NSC-71795) and Some Ellipticine Analogs [1,2,3]

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SUMMARY

The intravenous injection of ellipticine (12.0 mg/kg) into anesthetized rhesus monkeys was followed by a decrease in blood pressure, a decrease in heart rate, and an increase in carotid artery blood flow. In the isolated, blood-perfused dog heart ellipticine (12.0 mg) elicited an initial negative inotropic and chronotropic response followed in 5 minutes by secondary increases in these parameters above control levels. In most instances, these cardiovascular responses differed only quantitatively after the same dose of 7,10-dimethylellipticine, 9-methoxyellipticine, or 11-demethylellipticine. Ellipticine (12.0 mg/kg) or 7,10-dimethylellipticine (12.0 mg/kg), dissolved in HCl and adjusted to pH 4.0, was found to produce immediate and marked hemolysis in anesthetized rhesus monkeys. In contrast, the same dose of 9-methoxyellipticine or 11-demethylellipticine did not lyse red blood cells. Because of this lack of hemolytic activity these latter two compounds could ultimately prove to be of greater clinical value.

[Cancer Chemother Rep 58:637–643, 1974]

Ellipticine, an alkaloid isolated from the Australian plant *Excavatia coccinea*, exerts antitumor activity against certain experimental neoplasms (1) and has recently been proposed for clinical trials. Preclinical toxicity studies have revealed that intravenous (iv) administration of ellipticine produces alterations in the cardiovascular system (2,3). Hemolysis was also present immediately after the injection of ellipticine (4). In rhesus monkeys ellipticine (12.0 mg/kg) was found to produce marked hemolysis (5). Because of this potentially adverse reaction several structural analogs of ellipticine were made available for further study. The present study compares the acute hemolytic and cardiovascular actions of ellipticine with similar actions of three such analogs (fig 1).

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[2] Supported by contract NO1-CM-33728 from the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3] Ellipticine: CAS reg. No. 519-23-3; 6*H*-pyrido[4,3-*b*]carbazole, 5,11-dimethyl-. All drugs used in this study were supplied by Drug Research and Development, DCT, NCI.

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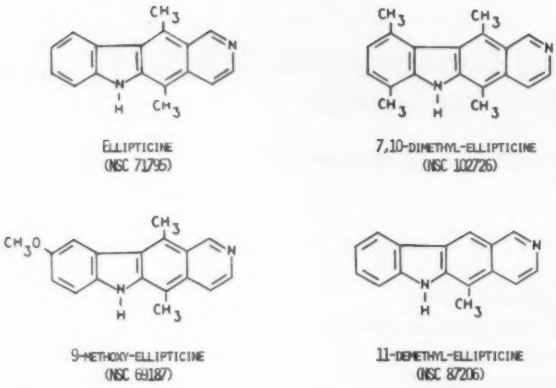

FIGURE 1.—Structures of ellipticine, 7,10-dimethylellipticine, 9-methoxyellipticine, and 11-demethylellipticine.

MATERIALS AND METHODS

Studies in Monkeys

Male rhesus monkeys (3.5–4.5 kg) were anesthetized iv with pentobarbital sodium (30.0 mg/kg) and secured to an operating table. Cannulas were inserted into the left femoral vein, left femoral artery, right femoral artery, and trachea. Each animal was given sodium heparin (1000 units/kg) via the femoral vein.

Ellipticine, 7,10-dimethylellipticine, 9-methoxyellipticine, or 11-demethylellipticine was infused iv at a dose of 12.0 mg/kg over a 4-minute period.[a] Each compound was initially dissolved in 1N HCl and the pH was adjusted to 4.0 with 2N NaOH. The final volume was adjusted to 8.0–10.0 ml with physiologic saline.

Hemolytic activity of ellipticine and analogs.—Each compound was given to three monkeys. One animal received the pH-4.0 vehicle. Before and 1, 5, 15, 30, and 60 minutes after injection, blood (4.0 ml) was collected from the right femoral artery directly into glass tubes. After 1 hour all samples were centrifuged ($760 \times g$) and the amount of hemolysis was determined by the benzidine method of Crosby and Furth (6). Briefly, the method involved mixing a portion of each plasma sample (0.1 ml) with 2.0 ml of benzidine reagent and 1.0 ml of 0.6% hydrogen peroxide in a glass tube. In those instances where considerable hemolysis had occurred the initial sample of 0.1 ml of plasma was diluted with 0.9 ml of physiologic saline and 0.1 ml of this diluted plasma was then mixed with benzidine reagent and hydrogen peroxide. After 60 minutes 20.0 ml of 20% glacial acetic acid was added to each tube and the color intensity was measured in a Beckman DB spectrophotometer at a wave-length of 515 nm. A standard derived from human red blood cells containing between 15 and 17 mg/100 ml hemoglobin was prepared fresh on the days the experiments were performed.

Cardiovascular activity of ellipticine and analogs.—Arterial blood pressure and respiration were monitored by means of a Hewlett-Packard 267 BC pressure transducer and a 270 pressure transducer and pneumotach screen connected to a Hewlett-Packard polygraph. Blood flow was measured by means of a Biotronex flow probe (2.0 mm) inserted around the right carotid artery and connected to a Biotronex electromagnetic flowmeter. The lead II electrocardiogram was obtained from subcutaneous needle-tipped electrodes and heart rate was determined by a cardiotach preamplifier. The parameters were continuously monitored in rhesus monkeys during the blood-sampling period.

Studies in the Isolated Dog Heart Preparation

Direct cardiac actions of ellipticine, 7,10-dimethylellipticine, 9-methoxyellipticine, and 11-demethylellipticine were studied in a series of isolated dog hearts. Adult mongrel dogs (10–12kg) were anesthetized with pentobarbital sodium and the hearts were isolated according to a previously described procedure (7). Force of contraction was monitored by means of a Walton-Brodie strain gauge. This strain gauge measures isometric systolic tension (8). The hearts were perfused in a volume of approximately 450 ml of blood and allowed to

[a] 7,10-Dimethylellipticine: NSC-102726; CAS reg. No. 21240-68-6; 6*H*-pyrido[4,3-*b*]carbazole, 5,7,10,11-tetramethyl-.

9-Methoxyellipticine: NSC-69187; CAS reg. No. 10371-86-5; 6*H*-pyrido[4,3-*b*]carbazole, 9-methoxy-5,11-dimethyl-.

11-Demethylellipticine: NSC-87206; CAS reg. No. 4238-66-8; 6*H*-pyrido[4,3-*b*]carbazole, 5-methyl-.

stabilize for 15–30 minutes before drug administration.

Once stabilized, the heart preparations were injected over a 2-minute period with 12.0 mg of ellipticine or one of the analogs. The compounds were dissolved in 1N HCl and back-titrated to pH 4.0 with 2N NaOH followed by physiologic saline to a final volume of 5.0 ml. In one experiment 5 ml of the pH-4.0 vehicle was administered. All experiments were terminated after 60 minutes. Each compound was repeated in three separate isolated heart experiments.

RESULTS

Hemolytic Activity of Ellipticine and Analogs

The iv infusion of 12.0 mg/kg of ellipticine increased the hemochromogen level from 14 to 334 mg/100 ml within 1 minute (table 1). Peak effects occurred during the first 15 minutes, after which the amount of hemochromogen decreased during each time interval. 7,10-Dimethylellipticine (12.0 mg/kg) produced equivalent amounts of hemochromogen over the same time course. In contrast, the average hemochromogen concentration was not significantly altered from the control level in animals given doses of 12.0 mg/kg of either 9-methoxyellipticine or 11-demethylellipticine. No increase in hemochromogen concentration over control levels was observed in the animal given 10.0 ml of the pH-4.0 vehicle only.

Cardiovascular Activity of Ellipticine and Analogs

A dose of 12.0 mg/kg of ellipticine decreased the mean arterial pressure by 58% (–40 mm Hg), decreased the heart rate by 10% (–14 beats/min), and increased the carotid artery blood flow by 50% within 1 minute of injection (fig 2). Blood pressure recovered completely within 5 minutes and remained at or near control levels for the duration of the observation period. The initial hypotension was less severe in monkeys given 9-methoxyellipticine or 11-demethylellipticine. Minor recovery occurred but was followed by a gradual decline such that at the end of 60 minutes mean arterial pressure was 76%–78% of the control level. 9-Methoxyellipticine caused a slight decrease in heart rate similar to that seen after treatment with ellipticine, whereas those animals given 11-demethylellipticine had a slight but persistent tachycardia. The initial decreases in blood pressure and heart rate were absent after 7,10-dimethylellipticine was given, although the secondary decline in blood pressure was comparable to that caused by the other two ellipticine analogs.

All compounds increased carotid artery blood flow ranging from 174% ± 17% (mean ± SE) after 11-demethylellipticine to 113% ± 2% after 9-methoxyellipticine. A rapid decline in this parameter occurred in all experiments within 5 minutes. After 60 minutes the flow

TABLE 1.—Comparison of the amount of hemochromogen (mg/100 ml) detected at various times after the injection of 12 mg/kg of ellipticine or its analogs in plasma from anesthetized rhesus monkeys

Time after injection (min)	Hemochromogen* (mg/100 ml)			
	Ellipticine	7,10-Dimethyl-ellipticine	9-Methoxy-ellipticine	11-Demethyl-ellipticine
Control	14 ± 6	17 ± 3	9± 6	9 ± 4
1	334 ± 56	475 ± 131	13 ± 9	13 ± 6
5	331 ± 65	391 ± 74	10 ± 7	9 ± 4
15	354 ± 15	334 ± 29	11 ± 8	10 ± 5
30	284 ± 20	280 ± 51	10 ± 6	8 ± 3
60	211 ± 11	249 ± 52	11 ± 6	6 ± 4

*Mean ± SE from 3 experiments.

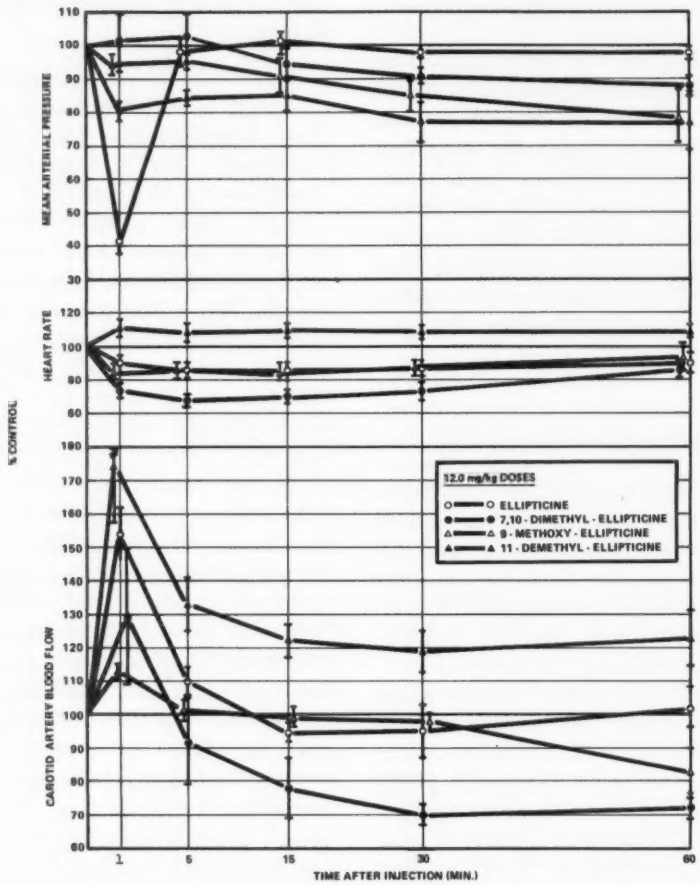

FIGURE 2.—Comparison of the effects of 4-min iv infusions of 12.0 mg/kg of ellipticine (○—○), 7,10-dimethylellipticine (●—●), 9-methoxyellipticine (△—△), or 11-demethylellipticine (▲—▲) on mean arterial pressure, heart rate, and carotid artery blood flow of rhesus monkeys. Values obtained were converted to percent control and each point represents the mean ± SE from 3 experiments.

was still significantly elevated in animals given 11-demethylellipticine (123% ± 8%) but was below control levels in animals given either 7,10-dimethylellipticine (72% ± 3%) or 9-methoxyellipticine (83% ± 7%).

The 4-minute infusion of ellipticine caused a decrease in amplitude of the R wave and a slight increase in depth of respiration. Similar effects were also seen after treatment with 7,10-dimethylellipticine which, in addition, caused an increase in the amplitude of the T wave. Both parameters returned to control levels by 5 minutes after injection. None of these effects were noted after the injection of either 9-methoxyellipticine or 11-demethylellipticine.

Isolated Dog Heart Experiment

The effect of doses of 12.0 mg of ellipticine and its analogs on the heart rate of the isolated dog heart is summarized in the top portion of figure 3. Ellipticine, 9-methoxyellipticine, and 11-demethylellipticine produced a significant increase in heart rate following a brief period of bradycardia. In contrast, 7,10-dimethylellip-

ticine produced a sustained decrease in heart rate for up to 60 minutes. The bottom portion of figure 3 demonstrates the effect of the same compounds on the force of contraction in the isolated heart. Except for 11-demethylellipticine, all agents produced an initial decrease in force of contraction. Ultimately this parameter was markedly increased by all compounds within 5–15 minutes. After 60 minutes this effect was still present in all hearts except those given 11-demethylellipticine. Pump perfusion pressure, not shown in figure 3 but recorded in all experiments, was depressed initially by 8%–20% but had completely recovered by 5 minutes. Five ml of the pH-4.0 vehicle had no observable effect on any of the parameters monitored.

DISCUSSION

Chemical agents can destroy red blood cells by at least three different modes of action: (*a*) by a direct interaction between cell and drug; (*b*) by a predisposition of the cell to damage

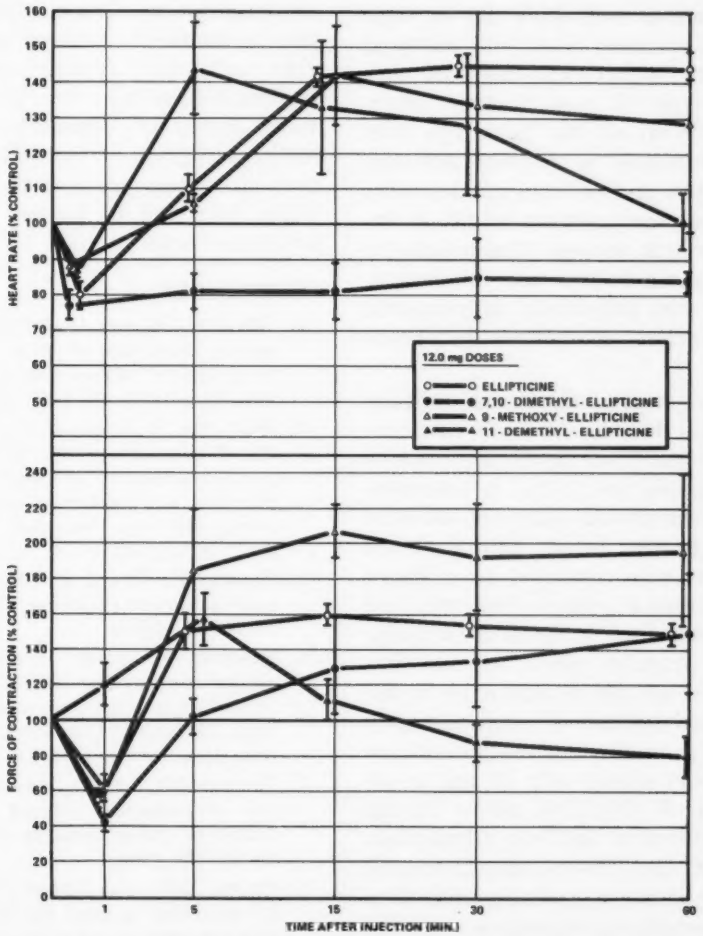
HEART RATE (% CONTROL)
FORCE OF CONTRACTION (% CONTROL)
TIME AFTER INJECTION (MIN.)
12.0 mg DOSES
ELLIPTICINE
7,10 - DIMETHYL - ELLIPTICINE
9 - METHOXY - ELLIPTICINE
11 - DEMETHYL - ELLIPTICINE

FIGURE 3.—Changes in heart rate and force of contraction at various times after administration of 12.0 mg of ellipticine (○—○), 7,10-dimethylellipticine (●—●), 9-methoxyellipticine (△—△), or 11-demethylellipticine (▲—▲) in the isolated, blood-perfused dog heart. Values obtained were converted to percent control with each preparation serving as its own control. Each point represents the mean ± SE from 3 separate experiments.

by its own inherent chemical abnormality; and (*c*) by formation of red cell antibody (9). Of the three modes, ellipticine and 7,10-dimethylellipticine appear to directly interact with the cell since both produced substantial hemolysis in the present in vivo experiments and in previously reported in vitro red blood cell preparations (10).

In these earlier in vitro studies ellipticine was taken up by the cell and rapidly lysed red blood cells at a 10^{-3} M concentration (10). The in vivo hemolytic activity detected in the present experiments correlates well with the in vitro findings that the greatest hemolysis occurred with 7,10-dimethylellipticine and the least with 11-demethylellipticine. It was further determined that the extent of the hemolysis correlated with differences in surface activity, lipophilic properties, and cellular uptake (10). These data suggest that calcium might be involved in the hemolytic process. The possibility exists that these same factors may be responsible for the observed differences in hemolytic activity of the various compounds in rhesus monkeys.

In the isolated heart, ellipticine initially caused a reversible decrease in both rate and force of contraction. This depressant action could partially explain the initial hypotension and bradycardia seen after iv administration in intact monkeys. In the intact animal, blood pressure and heart rate remained at or below control levels whereas a positive inotropic and chronotropic effect ensued in the isolated heart. These differences probably reflect the fact that in the 4-kg monkey 48 mg of ellipticine is distributed in a blood volume of approximately 400–500 ml which is similar to the blood volume of the isolated heart where only 12 mg is administered. Doses of ellipticine above 12 mg produce more depression and less stimulation of the isolated heart (3). The increase in carotid artery blood flow is most probably due to a decrease in total peripheral resistance.

In most instances, the cardiovascular responses were altered only quantitatively by the three ellipticine analogs. The initial hypotension was absent after treatment with 7,10-dimethylellipticine, whereas carotid artery blood flow increased to the greatest extent after treatment with 11-demethylellipticine. Thus, the initial cardiovascular effects do not appear to be correlated to a significant degree with the hemolytic activity of the compounds.

A similar lack of correlation appears to exist between hemolytic and antitumor activity. Ellipticine, which causes considerable hemolysis, increases the survival time of mice inoculated with L1210 tumor cells (1). Preliminary investigations indicate that 9-methoxyellipticine and 11-demethylellipticine exert a similar inhibitory activity in mice (Dr. I. Kline, personal communication). The ellipticine-induced hemolysis in monkeys occurred with the hydrochloride salt, but similar preparations of 9-methoxyellipticine or 11-demethylellipticine were devoid of hemolytic activity. Thus, 9-methoxyellipticine or 11-demethylellipticine may ultimately prove to be of greater value if either is found to possess equivalent clinical antitumor activity.

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Development of a Method for Assessing the Antitumor Activity of Chemotherapeutic Agents Using Human Tumor Xenografts [1,2]

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SUMMARY

Methotrexate, 5-fluorouracil, BCNU, and UC-51 were tested for activity against two human colonic carcinomas. The two tumors were grown as xenografts in immune-deprived mice. Immune deprivation was obtained either by thymectomy and whole-body irradiation or by thymectomy and treatment with antithymocyte serum. At the doses chosen, only 5-fluorouracil resulted in complete control of tumor growth. BCNU and methotrexate produced moderate reduction in tumor growth rate.

[Cancer Chemother Rep 58:645–651, 1974]

At present, the detection of antitumor activity in new drugs is based on the measurement of their cytotoxic activity against animal tumors. These test tumors are grafts of rapidly proliferating tumor cells usually growing in rats or mice, for example, the Walker 256 carcinosarcoma in rats and the L1210 leukemia in mice. Tumors in man bear only the vaguest histologic and cell kinetic resemblance to the transplantable rodent tumors, yet from an index calculated from observing the cytotoxic activity of a new drug against these animal tumors, we decide whether or not the drug is likely to be effective against cancer in man and, therefore, worthy of clinical trial.

In an attempt to prepare a more relevant "screen" for new antitumor agents, we are investigating the use of xenografted human tumors. It has been shown that human tumor can be grown in immune-deprived rodents and that it retains much the same histologic appearance and proliferating activity observed in the donor patient (1–3). This being so, it appeared reasonable to develop a screen for antitumor activity using human tumor growing in immune-deprived animals. This presentation explains the rationale and development of such a human tumor screen.

MATERIALS AND METHODS

Human Tumor

Two human tumors were used in the present study: well-differentiated (P116) and poorly differentiated (P76) carcinoma of the colon. Both tumors have been described in detail in a previous publication (3). Pieces of these tumors were originally implanted subcutaneously (sc) into immune-deprived mice within 1 hour of removal at hemicolectomy in the patients. The tumor material used for implantation in the present study was in the third, fourth, or fifth transplant generation. One piece of tumor, 20–30 mm^3, was implanted sc

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[5] We wish to acknowledge the technical assistance of Sylvia Clarke, Carolyn Palmer, and Norma Barron. We also wish to thank Professor Martin Tobe of University College, London, England, for supplying UC–51, and Dr. T. A. Connors for helpful discussions during the planning and execution of this work.

[6] *Reprint requests to*: Dr. Leon M. Cobb, Huntingdon Research Centre, Huntingdon, PE18 6ES, England.

in the right flank of each of 22 mice in each test.

Experimental Animals

Male CBA/lac mice were used. The mice were housed in metal boxes and bedded on sterile sawdust and wood-wool. They were fed Spratt's Laboratory Diet No. 1 (Lillico and Sons, Bletchworth, Surrey, UK) and given boiled water ad libitum.

Technique of Immune Deprivation by Thymectomy, Whole-Body Lethal Irradiation, and Bone Marrow Replacement

This technique was based on the work published by Miller (4) in 1963 in which the number of thymus-derived lymphocytes (T cells) is permanently and severely depleted. The mice were thymectomized at 4 weeks of age; 4 weeks later they were given 900 rads of whole-body irradiation. Irradiation was given at a rate of 60 rads/minute using a 200-kilovolt X-ray machine (half-value layer, 0.4 mm^3; focal distance, 100 cm). Without a bone marrow graft, this dose of irradiation would have been lethal. Within 6 hours of irradiation, each mouse received 5×10^6 syngeneic femoral bone marrow cells intravenously (iv). The tumor was implanted between 2 and 4 weeks after the irradiation and bone marrow replacement. For convenience of description mice prepared in this way are called irradiated mice.

Technique of Immune Deprivation by Thymectomy and Antithymocyte Serum

In this technique also, the mice were permanently depleted of T cells. They were thymectomized at 4 weeks of age and then treated with antithymocyte serum (ATS) from 6 to 9 weeks of age. ATS (0.5 cm^3) was injected sc in the interscapular region three times weekly for the 3-week period. The ATS was prepared in male New Zealand white rabbits. Two iv injections, each containing 5×10^8 mouse thymocytes, were given 2 weeks apart. The animals were bled out 7 days after the second injection. The bleeding and collection of serum were carried out under aseptic conditions to remove the necessity for filtering the serum. The rabbits were starved for 12 hours before bleeding out. They were anesthetized with sodium pentobarbital and the blood was collected from a cannula inserted into the abdominal aorta. The blood was allowed to stand for 1 hour at room temperature and was then centrifuged, and the serum was removed. After decomplementing in a water bath at 56 C for 30 minutes, the serum was divided into aliquots of 10 ml and stored at −20 C.

At the start of each experiment sample tubes of the serum from five rabbits were thawed, pooled, checked for sterility, and tested for immunosuppressive activity. For each of these tests, 0.5 cm^3 of the pooled serum samples from the five rabbits was injected sc into three mice three times weekly for 3 weeks, and 2 weeks after the last injection the human colonic tumor P116 was implanted sc. The colonic tumor for this test was harvested from immune-deprived mice where it was in the third, fourth, or fifth passage. The ATS was considered active if the implants (20–30 mm^3) of P116 grew to 1 cm^3 in 6–10 weeks. Inactive or low-activity serum was discarded and the active serum was further pooled so that the serum for the experiments was pooled from 25–30 immunized rabbits. For convenience of description, mice prepared by the above technique were designated ATS mice.

Drugs and Dosage

Three drugs that have been used for some years in clinical cancer chemotherapy were tested as well as a drug that is being considered for clinical trial. All four drugs were given at three dose levels. The highest dose level was that which could be administered to the irradiated mice without causing clinical abnormality or failure to maintain the same weight increase as that of the untreated group. In preliminary toxicology studies it was observed that the irradiated mice had increased sensitivity to chemotherapeutic agents. The implications of this are enlarged upon in the discussion. The two lower doses were one half and one quarter of the highest dose level. All drugs were given four times with 14-day in-

tervals between each dose. The drugs[7] and dose levels are as follows:

1. Methotrexate: The solvent control group received 0.4 ml of water for injection sc which was the volume of solvent used for the high-dose treatment. The treated animals received 10, 5, or 2.5 mg/kg of methotrexate per injection sc.

2. 5-Fluorouracil: The solvent control group received 0.4 ml of physiologic saline sc which was the volume of solvent used for the high-dose treatment. The treated animals received 200, 100, or 50 mg/kg of 5-FU per injection sc.

3. BCNU: The solvent control group received 0.4 ml of ethanolic saline sc which was the volume of the solvent used for the high-dose treatment. The treated animals received 10, 5, or 2.5 mg/kg of BCNU per injection sc.

4. UC-51: The solvent control group received 0.1 ml of arachis oil sc which was the volume of the solvent used for the high-dose treatment. The treated animals received 100, 50, or 25 mg/kg of UC-51 per injection sc.

Assessment of Antitumor Activity Using Irradiated Mice

The mice were immune deprived by the technique of thymectomy and whole-body irradiation with bone marrow replacement. In order to test a drug, tumor was implanted sc into 22 immune-deprived mice. They were randomly divided into five groups to be treated as follows:

Group I: six control animals receiving no treatment at all.

Group II: four solvent control animals receiving the volume of solvent used for the highest treatment dose.

Group III: four animals receiving the high-dose treatment.

Group IV: four animals receiving one half of the dose used in group III.

Group V: four animals receiving one quarter of the dose used in group III.

The first treatment was given 1 week after the implantation of tumor. Three further treatments were given at intervals of 2 weeks. Methotrexate, BCNU, and UC-51 were tested against the colonic tumors P116 and P76. Tumor volume was calculated by measuring the tumor along three diameters and multiplying the product of the three diameters by 0.6. This calculation gave a figure considered to be close to the real volume of the sc tumor. If the volume of a tumor grew to exceed 4 cm^3 the mouse was killed, because a tumor in excess of this volume could interfere with the general health of the animal and cause it to lose weight. All remaining mice were killed 12 weeks after implantation. Following implantation the animals were weighed weekly and 2 weeks after implantation weekly assessment of tumor was started. The effect of the drug on the colonic xenograft was assessed from plots of tumor volume against time.

Assessment of Antitumor Activity Using an Established Tumor in Irradiated Mice

Because of the occasional failure of the human tumor to "take," the above experiments were repeated except that treatment was delayed until 3 weeks after implantation, when the tumor had become established. The technique of tumor implantation, the grouping of the animals, and the measurement of tumor volume were exactly as in the previous section. The drugs tested against the established tumor were methotrexate, 5-FU, BCNU, and UC-51. An established tumor was one that had grown so that the sum of the two greatest diameters was 2 cm. As a result of this delay in giving the initial treatment, it was only possible to administer two or three treatments to the tumor-bearing mice before the conclusion

[7] Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl] methylamino]benzoyl]-. Supplied by Lederle Laboratories Division, American Cyanamid Co., Pearl River, NY.

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU. Supplied by Roche Ltd., Welwyn Garden City, Hertfordshire, UK.

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-. Prepared by Ben Venue Laboratories, Bedford, Ohio, for the Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

UC-51: NSC-170898; CAS reg. No. 38780-36-8; *cis*-dichlorobis(cyclopentylamine)platinum (II). Prepared under the direction of Professor Martin Tobe, University College, London, UK.

of the experiment 12 weeks after the implantation of the tumor.

Assessment of Antitumor Activity Using ATS Mice

Because there was a 3% mortality among irradiated mice, the tests were repeated using ATS mice which were constitutionally stronger. The mice were immune deprived by the technique of thymectomy and ATS treatment. The technique of tumor implantation and the grouping of the animals were exactly as described for the mice in the previous two sections. The colonic tumors P76 and P116 were both used. The drugs tested were methotrexate, 5-FU, BCNU, and UC-51 at the doses previously outlined.

RESULTS

Experiments Using Irradiated Mice

Methotrexate, UC-51, and BCNU were tested successfully against the two colonic tumors. Only BCNU had clear antitumor activity, which was shown against the poorly differentiated colonic tumor P76 (fig 1 [*a–e*]). Figures 1*a* and 1*b* illustrate the volume increase of tumors in untreated control and solvent control animals respectively. When these volume increases are visually compared with those of the same tumor during treatment with the high dose of BCNU (10 mg/kg), it is clear that the drug was depressing the growth of the tumor although the tumor growth was not completely suppressed. The body weight increase in these animals was the same as for the two control groups. The two lower doses of BCNU (5 and 2.5 mg/kg) did not affect the growth rate of the colonic tumor.

Methotrexate at the highest dose (10 mg/kg) showed activity against the colonic tumor P116 in two of the four implanted mice (fig 2*c*). Antitumor activity was not observed at the two lower doses of methotrexate (fig 2 [*d* and *e*]).

Figure 2*d* illustrates an important problem that arose with the irradiated mice, that is, there were occasional deaths. These deaths could not be related to the dose of the drug or the tumor volume and occurred equally in control and treated animals during the 12-week

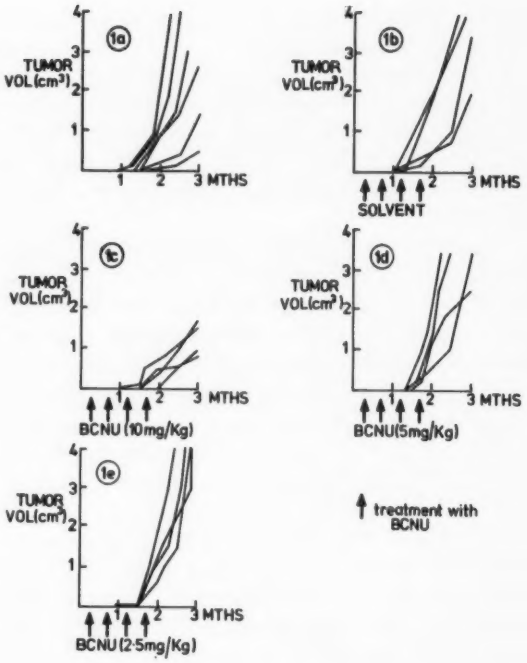

FIGURE 1.—Effect of BCNU on the human colonic tumor P76. The tumor was growing sc in immune-deprived mice. *a*: The change in tumor volume with time in 6 untreated mice. *b*: The change in tumor volume in 4 solvent control mice. *c*, *d*, and *e*: The changes in tumor volume as a result of 4 treatments with BCNU at 10, 5, and 2.5 mg/kg respectively. The solvent control and drug treatments were started 1 week after the implantation of the tumor. The animals were killed either when the sc tumor reached 4 cm³ or at the termination of the experiment at 12 weeks, whichever occurred first.

period of the experiment. Three percent of the irradiated mice failed to survive. Occasional deaths among mice after whole-body lethal irradiation and bone marrow replacement are encountered in other laboratories using such animals. The high level of irradiation necessary for immune suppression inevitably produces some damage in tissues other than the lymphocytes, for example, damage to the intestinal epithelium.

Results of Experiments Using Irradiated Mice and Delayed Treatment

Methotrexate, BCNU, 5-FU, and UC-51 were without apparent effect at the doses used

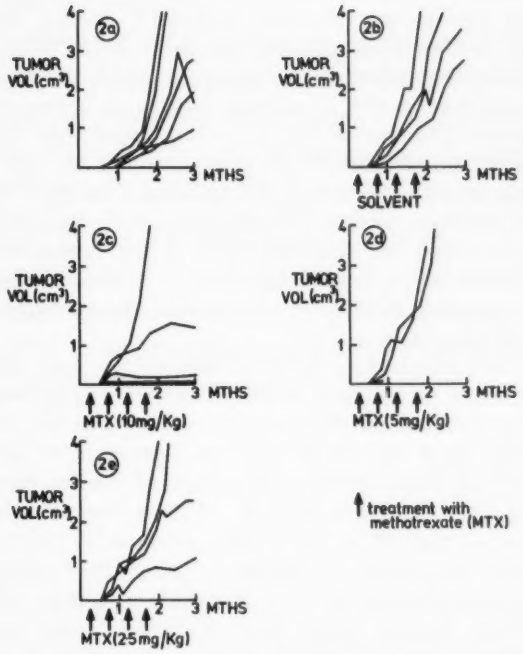

FIGURE 2.—Effect of methotrexate on the human colonic tumor P116. The tumor was growing sc in immune-deprived mice. *a*: The change in tumor volume with time in 6 untreated mice. *b*: The change in tumor volume in 4 solvent control mice. *c*, *d*, and *e*: The changes in tumor volume as a result of 4 treatments with methotrexate at 10, 5, and 2.5 mg/kg respectively. *d*: Two of the 4 mice in this group died during the treatment period. The solvent control and drug treatments were started 1 week after the implantation of the tumor. The animals were killed either when the sc tumor reached 4 cm^3 or at the termination of the experiment at 12 weeks, whichever occurred first.

against the colonic tumors P76 and P116 when they were administered 5–7 weeks after implantation.

Results of Experiments Using ATS Mice

The purpose of these experiments was to see if the occasional deaths observed in whole-body irradiated mice could be avoided by using ATS-treated immune-deprived mice. The result of the experiments was that sporadic deaths *were* avoided by using ATS mice. The ATS mice themselves were noticeably healthier than the irradiated mice.

The result of the tests for antitumor activity showed that *at the doses used* 5-FU was the most active of the four compounds.

The effective antitumor activity of 5-FU was shown against the well-differentiated colonic tumor P116 (fig 3 [*a–e*]). 5-FU at a dose of 200 mg/kg for four treatments at intervals of 2 weeks resulted in the total failure of the tumor to grow in all animals (fig 3*c*). At half the dose, two of the four mice showed no tumor growth and the other two mice showed limited growth of the tumor (fig 3*d*).

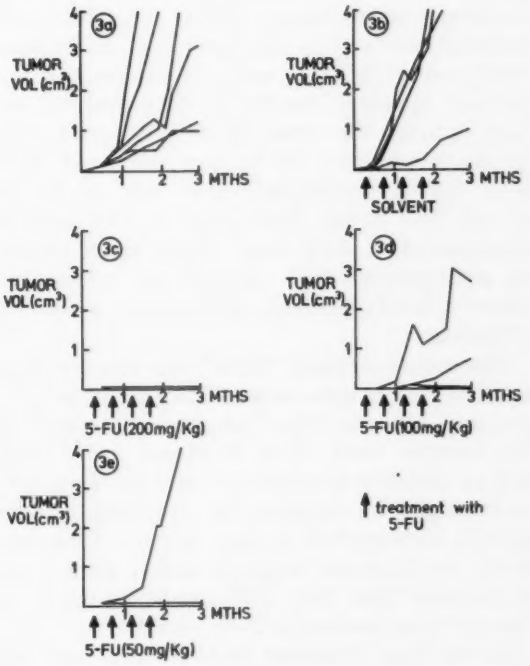

FIGURE 3.—Effect of 5-FU on the human colonic tumor P116. The tumor was growing sc in immune-deprived mice. *a*: The change in tumor volume with time in 6 untreated mice. *b*: The change in tumor volume in 4 solvent control mice. *c*, *d*, and *e*: The changes in tumor volume as a result of 4 treatments with 5-FU at 200, 100, and 50 mg/kg respectively. The solvent control and drug treatments were started 1 week after the implantation of the tumor. The only mice to die during the experiment were those killed because the volume of the tumor exceeded 4 cm^3. All other mice were killed at the termination of the experiment at 12 weeks.

DISCUSSION

This study was designed to investigate the possibility of using human tumor grafts in immune-deprived mice as a means of assessing the likely clinical value of new chemotherapeutic drugs. We feel that the above results indicate that such a screening test may be of value but that some modifications to the present technique are called for.

When heavily irradiated mice were used as donors, there were occasional deaths. The deaths did not cluster but occurred at all times throughout the 12 weeks of the test. There is little doubt that the basic cause of the deaths was irradiation, although the nature of the terminal disease varied. When the mice were immune deprived by the technique of thymectomy and ATS, there were no deaths and the animals appeared healthier. Subsequently, we have reduced the incidence of post-irradiation deaths to less than 1% by delaying irradiation until the thymectomized mice are 12 weeks of age. The tumor graft grew equally well in irradiated and ATS mice. However, it should be remembered that whole-body irradiation leaves behind persistent damage to a variety of tissues.

The mouse mutant "nude" can also be used as a host for human tumor. The animal is hairless and athymic (5). The congenital lack of the thymus leads to a deficiency in T cells and an inability to reject xenografts. Such animals might be suitable for screening drugs against xenografted human tumor. However, these are abnormal animals and it should not be assumed that they will metabolize drugs in the way that most other mice would.

It has been observed that the first four or five transplant generations of human tumor in a mouse closely resemble areas of the original tumor removed from the patient (3). It might be reasonable to expect from our experience with animal tumors that with repeated transplantation the cell population would lose its kinetic and histologic similarity to the original tumor. Because we are attempting to set up a screen with close relevance to the patient, we suggest that the testing should be carried out only on early transplant generations. However, because of the heterogenicity that is often found in the populations of tumor cells in a human primary tumor, we would expect that in any group of mice implanted during the early transplant generations there would be noticeable variations in tumor growth *between* mice and in the occasional failure of tumor to grow. Because of this it is suggested that the numbers of animals used per group in the present study were probably too small. The number of animals needed in each group will obviously vary with the heterogenicity of the tumor cell population, a smaller number of animals being used per group for the less heterogenous tumor cell populations.

The high dose level of the drugs was one quarter or one half of the dose that was known by us to affect the growth rate of the irradiated mice. It would have been possible to use higher doses, without drug-induced deaths, particularly in the ATS mice. Higher doses would have resulted in the tumor cells receiving, at least momentarily, levels of drug that could not possibly be attained in a patient. The translation of dose levels from mice to man has always been a complex question involving as it does differences in the pharmacokinetics of drugs in the two species. It is made more complex in the present situation where the tissue of one species is implanted into the other. Based on the evidence presented in this article it would not be possible to recommend one drug, for example 5-FU, in favor of another for the treatment of colonic carcinoma. Further evidence would be required over a wider range of dose levels.

It would be wise to regard a screen like the present one simply as a means of selecting drugs worthy of consideration for clinical trial and not as a means of comparing one drug with another in an attempt to identify the most suitable one for treating a particular patient.

The human xenograft screen is time-consuming and expensive but it is probably more relevant to man than the transplanted animal tumor screen. In relation to the total development cost of a new drug, the cost of the human tumor screen may be quite small. The development cost may be totally, and possibly unnecessarily lost if a drug is rejected

by a transplanted animal tumor screen of questionable relevance.

The screen described may only be of value for the selection of drugs suitable for treatment of carcinoma of the colon. Drugs used in the treatment of other types of tumors should be tested in screens using the relevant human tumors. At present we are studying human ovarian tumors and human bronchial tumors as xenograft screens for the selection of drugs likely to be effective against some cases of carcinoma of the ovary and bronchus.

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Phase II Study of Dibromodulcitol (NSC-104800) [1,2,3,4]

N. C. Andrews,[5] A. J. Weiss,[6] W. Wilson,[7] and T. Nealon [8,9]

SUMMARY

Dibromodulcitol (3.5 mg/kg/day) was administered orally to 181 patients with cancer who were acceptable for this study. Leukopenia (< 4500 cells/mm³) occurred in 70% of the patients and thrombocytopenia (< 150,000 cells/mm³) occurred in 75%. Objective responses were observed in 51 patients (28%). In 18 patients the objective response lasted 6 or more months and in eight patients it lasted 12 or more months. Two patients are still in remission 41 and 48 months, respectively, after the start of objective response.

[Cancer Chemother Rep 58:653–660, 1974]

Dibromodulcitol, like dibromomannitol,[10] is a halogenated sugar alcohol that acts biologically as an alkylating agent (1). The in vitro studies of Hidvégi et al (2) indicate that this drug inhibits DNA, RNA, and protein synthesis. Institóris et al (3) reported that dibromodulcitol has a biologic half-life of 4–5 hours. A recent study (4) of the absorption, distribution, and excretion of dibromodulcitol indicates that after oral administration the peak plasma level occurs at 2 hours and that the biologic half-life is 10 hours. The drug or its metabolites enter the spinal fluid and reach peak concentration between 6 and 8 hours.

Sellei et al (5) administered dibromodulcitol orally at a dose level of 5 mg/kg/day until leukopenia occurred. Of 50 patients studied, remissions were observed in 16 patients (32%). Toxicity was primarily myelosuppression and was reported to be relatively mild.

During a phase I study of dibromodulcitol (6), 96 evaluable patients received this drug in doses ranging from 0.25 to 5.0 mg/kg/day for 42 days. Twenty patients (20.7%) had objective remissions. These occurred in patients with the following types of carcinoma: breast, seven of 16; head and neck (lip), one of 13; stomach, three of four; kidney, one of five; and anus, one of one. Objective remissions were also seen in: Hodgkin's disease, two of five; melanoma, three of 12; sarcoma (fibrosarcoma), one of 15; and histiocytic neoplasm, one of one. Complete objective responses occurred in one patient with fibrosarcoma and in one patient with hypernephroma.

The toxicity noted during this phase I study was primarily myelosuppression manifested by

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[2] Supported by Public Health Service grant CA-12271 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3] This phase II study was conducted by the Central Oncology Group.

[4] Dibromodulcitol: CAS reg. No. 10318-26-0; galactitol, 1,6-dibromo-1,6-dideoxy-. Supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI.

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[8] New York University St. Vincents Hospital and Medical Center of New York, NY.

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[10] Dibromomannitol: NSC-94100; CAS reg. No. 488-41-5; 1,6-dibromo-1,6-dideoxy-D-mannitol.

leukopenia and/or thrombocytopenia. One drug-related death occurred from infection in a patient with severe leukopenia.

MATERIALS AND METHODS

Based upon the experience of the phase I study, the Central Oncology Group developed a phase II protocol for the study of dibromodulcitol which was to be administered orally for 42 days at a dose level of 3.5 mg/kg/day based on ideal or actual weight, whichever was less.

Patients admitted to this phase II study were required to have a tissue diagnosis of cancer. The investigators were discouraged from admitting patients who were over 70 years of age, had received pelvic irradiation, had extensive metastases to the bone, or had marrow damage from previous chemotherapy. Patients were not acceptable if they had damaged bone marrow, renal disease, poor protein intake, severe jaundice, or if they were in the terminal stages of disease.

Dibromodulcitol was discontinued if the white blood cell count (wbc) fell below 4000 cells/mm^3, if the platelet count fell below 100,000 platelets/mm^3, or if any evidence of hazardous toxicity developed during therapy. Upon recovery from toxicity the drug could be cautiously reinitiated. Hematologic evaluation of each patient was obtained weekly and other study parameters were obtained at specified intervals. The malignancies to be studied were carcinoma of the breast, gastrointestinal tract, kidney, lung, and head and neck; lymphoma; melanoma; and sarcoma. Measurable malignant lesions were required in all patients admitted to the study.

Two hundred and three patients were registered. Twenty-two were classified as unsatisfactory because of inadequate measurements of known lesions, insufficient medication, or both. Thus, of the patients registered, 181 were acceptable study patients. Information concerning the 181 study patients is given in tables 1 and 2.

Duration of Treatment

The protocol specified that dibromodulcitol should be given at a dose level of 3.5 mg/kg/day for 42 days. Sixty-three patients (34%) received the drug as recommended (table 3). Twenty-two patients received the drug for longer than 46 days, and 78 patients received dibromodulcitol for less than 40 days. Hematologic toxicity was the usual reason for early discontinuation of the drug. Actual or anticipated response in a patient accounted for the extended period of treatment.

TABLE 1.—Patient information

Total No. of patients	181
Age (yrs)	
Range	19–84
Mean	55.6
Sex	
Men	88
Women	93
Risk classification	
Good	137
Fair	28
Not stated	16

TABLE 2.—Therapy received prior to treatment with dibromodulcitol

Type of prior therapy	No. of patients
Resectional surgical treatment	132
Radiation therapy	65
Chemotherapy	108
Alkylating agents alone	22
Antimetabolites alone	21
Miscellaneous agents	15
Chemotherapeutic agents in combination	50

Toxicity

The two major toxic manifestations of dibromodulcitol were leukopenia and thrombocytopenia. For the purpose of this study, leukopenia was defined as any fall in the wbc to below 4500 cells/mm^3, and thrombocytopenia was defined as a fall in the platelet count to below 150,000 platelets/mm^3. A fall in the wbc occurred in 127 (70%) of the patients treated (table 4), and a fall in the platelet count occurred in 131 (75%) (table 5). Infection developed in seven patients with leukopenia. Of these seven, four had pneumonia, one had a urinary tract infection, one had an upper respiratory infection, and one had a wound

infection with dehiscence of the wound. Three patients had bleeding associated with thrombocytopenia, manifested as epistaxis in one, gastrointestinal bleeding in one, and from an unspecified source in the third.

Nausea and vomiting occurred in approximately one sixth of the patients but was relatively easily controlled with antiemetics. Toxic effects of dibromodulcitol are detailed in table 6.

RESULTS

Malignant lesions treated with dibromodulcitol are reported in table 7.

Results of treatment were evaluated by measurement of known malignant lesions which were examined by the investigators at weekly intervals. If more than one lesion was present, they were counted and the number was recorded. The following criteria were used in evaluating measurable lesions:

complete response—disappearance of all disease;

partial response—more than a 50% regression of the measurable lesion(s);

mixed response—more than a 50% regression of some disease with progression of less than 50% of other lesions;

no change—less than 50% response of any lesion and less than 100% increase in any lesion (50% doubling rule);

TABLE 3.—Duration of therapy with dibromodulcitol

Duration of therapy (days)	Total No. of patients
7–19	5
20–29	34
30–39	39
40–44*	82
45–54	9
55–154	13

*42 Days = 63 patients.

TABLE 6.—Dibromodulcitol toxicity

Toxic effect	No. of patients
Leukopenia <4500 cells/mm³	127
Thrombocytopenia <150,000 cells/mm³	131
Leukopenia and thrombocytopenia	26
Nausea and vomiting	40
Dyspnea	19
Serum glutamic oxaloacetic transaminase elevation	13
Anemia (drop of 4 g/100 ml Hgb)	11
Infection	7
Upper respiratory	2
Pneumonia	2
Urinary	2
Cellulitis	1
Nausea	5
Hemorrhage	4
Gastrointestinal	1
Epistaxis	1
Unspecified	2
Anorexia	3
Diarrhea	2
Pigmentation of skin	2
Pruritis	1
Vertigo	1
Blood urea nitrogen elevation	1

TABLE 4.—Toxicity: leukopenia

Wbc (cells/mm³)	Total No. of patients	Days of treatment (mean)	Day of nadir (mean)	Day of onset (mean)	Mean duration (days)
3000–4500	47	44	38	31.6	17
2000–2999	37	37	47	27	36
1000–1999	35	35	44	22	58
500–999	7	33	44	26	36
300	1	32	58	28	87

TABLE 5.—Toxicity: thrombocytopenia

Platelet count (platelets/mm³)	Total No. of patients	Days of treatment (mean)	Day of nadir (mean)	Day of onset (mean)	Mean duration (days)
75,000–150,000	83	42	37	29	17
50,000–74,000	23	41	45	23	23
5,000–49,000	25	35	38	19	27

TABLE 7.—Malignant lesions treated with dibromodulcitol

Malignancy	No. of patients	No. of responses	Partial or mixed responses	Complete response	Duration of response (mos)	Mean duration of response (mos)	Median duration of response (mos)
Breast	33	13			2–18	6	4.5
Adenocarcinoma	30	12	11	1	2–18	6.5	4.8
Ductal carcinoma	3	1	1		3		
Lung	37	8			1–9	3.0	3.0
Adenocarcinoma	11	2	2		1 and 1½	1.0	1.0
Epidermoid carcinoma	14	2	2		2½ and 9	5.8	5.8
Undifferentiated carcinoma	7	2	2		3 and 3	3.0	3.0
Bronchiolar carcinoma	1	0					
Histology unspecified	4	2	2		3 and 4	3.5	3.5
Stomach	2	0					
Colon	22	3	3		1, 3½, 11	5.2	3.5
Rectum	5	1		1	3		
Anus	1	1		1	35		
Head and neck	12	3			1–48		
Tongue	2	2	2		1 and 1½	1.2	1.2
Buccal membrane	2	1		1	48*		
Nasal mucosa	2	0					
Ethmoid sinus	1	0					
Tonsil	2	0					
Alveolar ridge	1	0					
Salivary gland	1	0					
Larynx	1	0					
Kidney							
Adenocarcinoma	6	1	1		3½		
Transitional cell	1	1	1		3½		
Bladder	1	0					
Prostate	1	0					
Fallopian tube adenocarcinoma	1	1		1	41*		
Ovary adenocarcinoma	3	1	1		18		

Carcinoma primary unknown	4	2			4 and 8	6.0	6.0
Epidermoid carcinoma	1	1	1		8		
Undifferentiated carcinoma	1	1	1		4		
Histology unspecified	2	0					
Sarcoma	18	3				4.3	4.0
Fibrosarcoma	1	1	1		1		
Liposarcoma	4	1	1		4		
Spindle cell sarcoma	1	1	1		8		
Neurosarcoma	2	0					
Synovial cell sarcoma	2	0					
Osteogenic sarcoma	2	0					
Leiomyosarcoma, uterus	2	0					
Angiosarcoma	1	0					
Ewing's sarcoma	1	0					
Unspecified type	2	0					
Melanoma	19	7	6	1	1½–8	4.1	3.5
Lymphosarcoma	4	3	2	1	8–36	17.7	9.0
Lymphoma	3	2	2		5–30	17.5	17.5
Hodgkin's disease	3	0					
Thymoma	1	0					
Plasmacytoma	1	0					
Cylindroma	1	0					
Lymphatic leukemia	1	0					
Carcinoid	1	1		1	6		
Total	181	51	43	8	1–48	8.4	4.5

*Continuing response.

progression—more than 100% growth in one or more lesions or the development of new lesions.

Forty patients with measurable lesions had partial responses and two had mixed responses. Complete responses occurred in nine patients. Thus, 51 (28%) of the 181 patients had either a partial, mixed, or complete response (table 7). Ninety-five patients were considered to have manifested no change in their measurable lesions, and 35 had progression. There was no relationship between the development of toxicity and response of the malignant disease to dibromodulcitol.

While not an accepted criterion of the effect of a study drug by the Central Oncology Group, it is of interest that 15 of the 95 patients classified as manifesting no change had a reduction in their measurable lesions of between 25% and 45% of the pretreatment size, and 27 of the 95 patients had either a lesion that did not increase in size or that decreased in size to less than 25% of its pretreatment measurement.

Duration of Therapy and Response

Dibromodulcitol was administered for an average of 33 days before a 50% decrease in the size of a measurable malignant lesion occurred. For patients with carcinoma of the breast and melanoma, the average time for response was 34 days; for those with carcinoma of the lung, 40 days; and for those with carcinoma of the colon, 41 days. Patients with carcinomas involving the head and neck responded early (average, 24 days), as did the several patients with sarcomas (average, 28 days). One patient with carcinoma of the lung received 62 days of treatment before a 50% response of the measurable lesion occurred.

DISCUSSION

Phase I and II studies of dibromodulcitol have been done by the Central Oncology Group. In the phase I study, leukopenia, thrombocytopenia, or both occurred in 68% of 96 patients. In the phase II study, similar hematologic suppression occurred in 86.7% of 181 patients. This difference in occurrence is explained by the fact that in the phase I study the patients in whom either leukopenia or thrombocytopenia developed had received dibromodulcitol at doses higher than 2 mg/kg/day; a significant number of patients received the drug at levels of from 0.25 to 2 mg/kg/day.

The incidence of hematologic toxicity in the present study is much higher than that reported by Sellei et al (5) in a study of 50 patients, 35 of whom had solid tumors. Leukopenia (< 4000 cells/mm^3) developed in only seven patients, and four patients had platelet counts below 100,000 cells/mm^3 despite administration of dibromodulcitol at a dose level of 5 mg/kg/day. The drug was continued until evidence of leukopenia developed. Since the criteria for discontinuation of dibromodulcitol were essentially the same in both studies, the difference in hematologic toxicity might be explained in part by the difference in defined levels of toxicity, ie, a wbc of 4500 versus 4000 cells/mm^3, and a platelet count of 150,000 versus 100,000 platelets/mm^3. Other explanations for the difference might be patient selection, prior or concurrent drug therapy, or radiation therapy. It is unlikely that a difference in drug potency would be a factor in view of the similarity of end points of discontinuation of the drug.

Keyes et al (7) administered dibromodulcitol orally at doses of 100 mg/m^2 (seven treatment courses), 130 mg/m^2 (five treatment courses), and 200 mg/m^2 (four treatment courses). Leukopenia and thrombocytopenia developed in one of three patients receiving the lower dose, in four of five patients receiving the intermediate dose, and in four of four patients receiving the 200-mg/m^2 dose. Three of the latter patients died during the period of pronounced hematologic toxicity.

Gevai et al (8), in a study of 82 patients treated with dibromodulcitol, report that severe thrombocytopenia occurred in four patients requiring discontinuation of the drug. Whether these four include one patient who died as the result of agranulocytosis and thrombocytopenia is not clear. Since the 82 patients included in this study were given transfusions of 320–400 ml of whole blood four or five times a week despite the peripheral blood picture, the minimal incidence of thrombocytopenia and leukopenia is probably attributable to this supportive therapy.

In 1969, Sellei et al (5) reported their experience with dibromodulcitol in 60 patients. Of these patients, 50 were evaluable and 35 had solid tumors. These authors reported objective remissions in six patients with solid tumors, ie, four of eight with upper respiratory tract cancers, one of six with bronchial carcinomas, and one of four with mammary cancer. In addition, two of five patients with Hodgkin's disease had objective remissions, as did eight of eight patients with chronic myelogenous leukemia.

In a later review, which apparently included some of the patients previously reported, Sellei et al (9) reported that of 132 evaluable patients treated with dibromodulcitol, 23 were treated successfully.

Keyes et al (7), in a study of 22 patients, found no evidence of therapeutic effect of dibromodulcitol. This observation may be explained by the selection of patients with relatively unresponsive tumors and the small number of patients treated.

Gevai et al (8) have reported objective improvement in 13 of 68 patients with proven primary bronchial carcinomas. Since these authors fail to indicate their criteria for an objective remission other than "as established by cancer tissue remission and X-ray morphology evidence," it is difficult to evaluate these reported results. Possibly a better indication of the effectiveness of dibromodulcitol is the increase in survival of patients with confirmed bronchogenic carcinoma who were treated as compared to those who received no treatment. In 46 patients with carcinoma of the lung who were treated with dibromodulcitol, the survival time was 294 ± 38 days, while in 46 control patients with carcinoma of the lung the survival time was 209 ± 39 days. A potential criticism of this criterion is that the control patients, while matched to the treatment patients by numbers, age, carcinoma type, and location, were observed prior to the development of "cytostatical treatment."

Ohnuma et al (10) have used dibromodulcitol at a dose of 3 mg/kg/day for 6 weeks in combination with bleomycin (NSC-125066) at a dose of 15 mg/m^2 given twice weekly to patients with squamous cell carcinoma originating in various organs. While they noted an objective regression of measured lesions of 50% or more in five of 20 patients with squamous cell carcinoma of the head and neck and in one of two patients with carcinoma of the skin, these authors conclude that the effectiveness of this therapeutic combination was primarily attributable to bleomycin and that dibromodulcitol did not appear to increase the response rate. They report that leukopenia ranging from 150 to 3700 cells/mm^3 with a median nadir of 2900 cells/mm^3 occurred in 18 of 29 patients and was often associated with thrombocytopenia. Dibromodulcitol was assumed to be the cause of this toxicity and one drug-related death.

As indicated earlier, during this phase II investigation of dibromodulcitol, objective remissions were observed in 51 of the 181 patients in the study. The duration of these responses was 3 or more months in 38 patients, 6 or more months in 19 patients, and 12 or more months in eight patients. Of those patients having remissions that lasted 6 or more months, the primary malignancies were: adenocarcinoma of the breast (five), ovary (one), fallopian tube (one), and colon (one); epidermoid carcinoma of the lung, buccal membrane, anus, and unknown primary (one each); lymphosarcoma (three); nonHodgkin's lymphomas (one); melanoma (one); carcinoid (one); and spindle cell sarcoma (one).

While the overall response rate in this study was 28%, the remission rate approached 40% in patients with carcinoma of the breast. Eight of the 12 responding patients continued in remission for 4 or more months. The number of patients included who had malignancies originating in other primary sites were too few to calculate a response rate. However, several observations should be emphasized. Dibromodulcitol does appear to produce responses in some patients with carcinoma of the lung, and in at least one patient with an epidermoid carcinoma the remission lasted for 9 months. While the length of remission was not as long in patients with undifferentiated carcinoma of the lung, the response rate tended to be slightly higher than in patients with adenocarcinoma or epidermoid carcinoma of the lung.

The length of remission in the single patients

with epidermoid carcinoma of the anus and of the buccal membrane, both of whom had complete responses, is noteworthy as is the fact that the patient with the oral lesion continues in remission. Similarly, the prolonged remissions of the patient with an adenocarcinoma of the ovary and of the patient with adenocarcinoma of the fallopian tube who continue as complete responses are notable. This agent might also prove useful in the treatment of lymphosarcoma and nonHodgkin's lymphoma.

The toxic effect of dibromodulcitol on the bone marrow at the dose level and duration used in this investigation is a limiting factor to its use. Two possible alternatives might be considered. One alternative would be to administer dibromodulcitol at a lower dose, since in the phase I study done by the Central Oncology Group one third of the patients who attained a remission received dibromodulcitol in doses that ranged between 0.25 and 3 mg/kg/day for 42 days. A second alternative would be to administer dibromodulcitol weekly or biweekly. A. J. Weiss (personal communication) is currently investigating the tolerance and effectiveness of dibromodulcitol when administered at a dose level of 1.2 g/kg at weekly intervals. Preliminary evaluation indicates that toxicity and therapeutic effectiveness are similar to those reported in this phase II study.

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Daunorubicin (NSC-83142) in Advanced Childhood Leukemia [1,2,3]

Norman Jaffe, Demetrius G. Traggis, and Lakshmi Das [4,5]

SUMMARY

Daunorubicin was administered to 44 children with acute lymphoblastic leukemia and to nine children with acute myelogenous leukemia in courses of 1 mg/kg/day for 3 consecutive days. This regimen was repeated after 4-day rest periods. Forty-two patients also received concurrent prednisone (1 mg/kg/day) in a divided dose. A minimum of two courses of daunorubicin was required for evaluation. Of the patients who were evaluated, there were five remissions among 40 patients with acute lymphoblastic leukemia and two remissions among nine patients with acute myelogenous leukemia. Periods of bone marrow hypoplasia preceded responses in all cases. Twenty-eight patients died from hemorrhage, or sepsis, or both within the first 2 months of treatment. The duration of remissions varied from 3 weeks to 2½ months. Daunorubicin has a therapeutic effect in advanced leukemia in children; alternative doses and schedules should be investigated to improve its potential and to decrease its toxicity.

[Cancer Chemother Rep 58:661–665, 1974]

Several studies have documented the efficacy of daunorubicin in the treatment of leukemia in children (1–13). In clinical studies, a variety of doses and schedules have been employed which produced responses from approximately 18% to 84% of previously treated patients. Prednisone appeared to enhance the frequency of remission induction in some investigations (2,6,10,11) but not in others (12). In 1970, the Children's Cancer Research Foundation undertook a study to determine the therapeutic effectiveness of daunorubicin in advanced leukemia in children who were refractory to all conventional chemotherapeutic agents. We describe our experience and review previous reports.

MATERIALS AND METHODS

Forty-four children with acute lymphoblastic leukemia (ALL) and nine children with acute myelogenous leukemia (AML) were studied. All patients were in bone marrow relapse subsequent to prior treatment with methotrexate, 6-mercaptopurine, vincristine, prednisone, cyclophosphamide, and cytosine arabinoside. Details of the patients are shown in table 1.

Courses of daunorubicin (1 mg/kg/day) were administered for 3 consecutive days and repeated after 4-day rest periods. At the first sign

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[2] Supported in part by Public Health Service grant CA-6516 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3] All drugs used in this current study were supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI. Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

[4] Children's Cancer Research Foundation, Inc., Boston, Mass.

[5] *Reprint requests to*: Norman Jaffe, MD, Administrative Chief, Out-Patient Tumor Therapy Clinics, Children's Cancer Research Foundation, Inc., 35 Binney St, Boston, Mass 02115.

TABLE 1.—Response and hematologic toxic effects of patients with acute lymphoblastic and acute myeloblastic leukemia

Disease	No. of patients evaluated	Age range (yrs)	Duration of disease (yrs)	No. of patients receiving concurrent prednisone therapy	Total No. of remissions*/ No. of remissions associated with prednisone	Duration of remissions (wks)	Amount (mg/kg) of dauno-rubicin needed for remission (range)	Maximum dose (mg/kg) of dauno-rubicin	Blood cell counts	
									Lowest: platelets/ wbc (cells/ mm³)	Nadir: platelets/ wbc (days)
Acute lymphoblastic leukemia	40	2½–12⅙	¾–5½	33	5/4 (4 CR, 1 PR)	3–10	6–19	27	0/100	22/22
Acute myeloblastic leukemia	9	4⅚–15⅓	½–4¾	9	9/2 (2 PR)	6–8	6–14.5	14.5	600/100	7/16

* CR = complete remission; PR = partial remission.

of severe bone marrow hypoplasia, chemotherapy was interrupted until recovery occurred. If excessive infiltration of blast cells was observed, chemotherapy was continued (despite bone marrow hypoplasia) with appropriate reduction in dosage (generally 50% of the previous dose). Prednisone (1 mg/kg/day) was administered in a divided dose. It was omitted in seven patients who were extremely cushingoid after a recent treatment with this drug. At least two courses of daunorubicin were required for evaluation.

Prior to entering the study, all patients were given physical examinations, bone marrow aspirations, complete blood cell counts (including platelet counts), liver function tests, and electrocardiograms (EKGs). Bone marrow examinations were repeated before the third and subsequently alternate courses of treatment, or as indicated by cytopenia. EKGs were repeated at frequent intervals, and hemograms were taken at least three times per week. Other investigations were conducted as dictated by the clinical status of the patients. Blood and platelet support were liberally provided and infections were treated vigorously with appropriate antibiotics.

A complete remission was defined as an adequately cellular bone marrow with a blast count of under 5%, and a partial remission was defined as a blast count between 5% and 10%. The study was terminated after the third course of treatment for patients who did not respond and was continued in all patients if there was a suggestion of improvement. It was anticipated that the maximum cumulative dose of daunorubicin would be 25 mg/kg. Patients entering remission received maintenance therapy with single chemotherapeutic agents that appeared to have produced maximum prior response.

RESULTS

Four patients with ALL died from sepsis before completing the requirements for evaluation. Of the remaining 40 patients, 33 received concomitant prednisone. There were five remissions (four complete and one partial). The four complete remissions were obtained with a combination of prednisone and daunorubicin. The doses of daunorubicin required to produce remissions ranged from 6 to 19 mg/kg. The maximum dose administered (27 mg/kg) without prednisone failed to induce a response. Each remission was preceded by bone marrow hypoplasia (total nucleated cell count under 50,000 cells/mm^3) and was followed by a regeneration of bone marrow in 7–10 days. The median time for remission induction was 24 days and the duration of remission varied from 3 weeks to 2 months. The bone marrows of patients who failed to achieve a remission were characterized by persistent infiltration with blast cells or hypoplasia after treatment and repopulation with leukemic cells.

All nine patients with AML were evaluated; eight received concurrent prednisone resulting in two partial remissions. The doses of daunorubicin required to achieve remission were 6 and 14.5 mg/kg, respectively, after brief intervals of pancytopenia. The duration of the remissions was 6 weeks and 2 months, respectively.

Toxic Effects (table 1)

Three children experienced mild stomatitis. There were no EKG abnormalities or episodes of cardiac failure. The principal toxic effect was bone marrow hypoplasia, predisposing to infection and hemorrhage. Leukocyte counts under 500 cells/mm^3 occurred in 11 patients and values between 500 and 1000 cells/mm^3 occurred in 24 patients. Thirty-seven patients had platelet counts under 50,000 platelets/mm^3. In ALL, the lowest platelet and white blood cell (wbc) counts recorded were 0 platelets/mm^3 and 100 cells/mm^3, with the nadir in both instances occurring at 22 days. In AML, the lowest platelet and wbc counts were 100 platelets/mm^3 and 600 cells/mm^3, with the nadirs at 7 and 16 days, respectively. Twenty-eight children died within 2 months of the start of treatment from overwhelming disease with hemorrhage, or sepsis, or both.

DISCUSSION

Daunorubicin has been effective in a number of clinical trials in children with advanced leukemia in different doses and schedules. In their studies, Jacquillat et al (1) and Massimo

et al (2) used doses of 1 mg/kg/day for 5 days resulting in remission rates of 50% and 87%, respectively. Among the patients reviewed by Tan et al (3), five of 13 (38%) patients entered good partial remissions when administered similar doses and additional therapy of 1–7.5 mg/kg after a 3-day rest period. Bernard (4) employed a dose of 30 mg/m² for 3–7 days, and a second course of treatment of 10 mg/m² for 1–4 days (if there was no response). This produced a 61% complete and partial response (22 of 36 patients). Remission rates of 18%, 33%, and 38% were reported by Jones et al (5) in patients who received a full course of treatment at 30, 35, and 60 mg/m²/day, respectively, for 5 days. The response rate was much lower (18%) in a study with 125 patients reported by Shore (13) who used 0.5–1 mg/kg/day to a total dose of 6–12 mg for 3 days with a 4-day rest period. The overall response rate in these studies is about 40%.

The contribution of prednisone to the ultimate response rates in patients receiving this agent and daunorubicin is difficult to assess. In the studies reported by Massimo et al (2), three of six patients with a complete remission had severe drug-induced marrow aplasia that was treated with prednisone and testosterone. Howard and Tan (6) observed an M_1 marrow in five of seven patients resistant to conventional chemotherapy who were treated with both drugs. Holton et al (10) reported four complete and three partial remissions among 39 patients who received 25 mg/kg/day for 3 days and after a 4-day rest period. Four of the seven responders received concomitant steroid therapy. These investigators concluded that daunorubicin had low-grade activity for remission induction in advanced leukemia and that combined with prednisone it may enhance the responses. In a later study, Holton et al (11) observed 49 complete or partial remissions in 60 children with advanced leukemia (82%). When patients who had had one previous course of prednisone were eliminated, a 57% remission rate was computed (17 of 30). This compared favorably with the 57% remission rate reported by Jones et al (12) in patients also treated with the two-drug combination. The latter authors concluded that there was no therapeutic advantage in combining daunorubicin with prednisone (or with prednisone and vincristine).

In our series there were seven responses; six of these were obtained with concurrent prednisone. These six patients had received two to six previous trials with prednisone in combination with other agents. Their responses were inconsistent and they were considered to be refractory to this chemotherapeutic agent when entered into the study. It appeared unlikely that prednisone significantly contributed to remission induction; the absence of response in the 36 other patients also treated with these two chemotherapeutic agents supports this assumption. No clear-cut differences between responders and nonresponders were discernible with respect to age, wbc count at initiation of treatment, and organ infiltration.

The overall response rate (14%) and the duration of remissions in the present study, though similar to that reported by Shore (13) and Jones et al (5) in one of their groups, are disappointing. The many variables in patients with advanced disease make comparison with other studies extremely difficult. In our patients nutrition was poor, anorexia was prominent, many organs exhibited leukemic infiltration, and sepsis, hemorrhage, and electrolyte disturbances were frequent complications. The picture was further complicated by severe life-threatening hematologic toxicity induced by daunorubicin in patients with an already poor bone marrow reserve.

It is to be emphasized that the response rate in previously untreated patients may be considerably higher. In a few published reports using daunorubicin alone, the response rate varied from approximately 60% to 100% (2–4,7). Also, this drug is highly effective in the treatment of myelogenous leukemia with a therapeutic potential equal to or probably superior to any other agent, both in the early and advanced stages (8,9). In our series, two of the nine patients with AML responded compared to five of the 40 patients with ALL.

We conclude that daunorubicin is effective in advanced refractory leukemia in children. Since the results were achieved at a serious

cost of morbidity and mortality, and since the durations of remissions were brief, alternative doses and schedules should be investigated.

LIST OF COMPOUNDS

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride

Daunorubicin: CAS reg. No. 23541-50-6; daunomycin (NSC-82151)

6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]-methylamino]benzoyl]-

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

Testosterone: NSC-9700; CAS reg. No. 58-22-0

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Phase II Study of CCNU (NSC-79037) in the Treatment of Advanced Gastrointestinal Cancer [1,2]

David J. Klaassen and Edna Rapp [3,4]

Disseminated gastrointestinal cancer is generally considered to be relatively unresponsive to cancer chemotherapeutic agents. 5-Fluorouracil [5] is the only agent that regularly produces remissions, and most authors report a 10%–30% objective response rate with this drug (1–3). Remissions, once obtained, are generally of short duration (usually less than 6 months). Consequently, newer agents are constantly being evaluated for activity in this group of malignancies.

CCNU is one of the nitrosoureas (4) that has been shown to produce remissions in this group of cancers. In one study, objective tumor regressions were noted in six of 25 patients (5). The dosage used was 130 mg/m² at 8-week intervals. Toxic effects were nausea, vomiting, and late bone marrow depression. Based on these results, we set out to evaluate this compound in a phase II study of a variety of patients with advanced gastrointestinal malignancies.

PATIENTS AND METHODS

Patients were considered for this study if they had:

a. biopsy-proven disseminated gastrointestinal cancer;
b. measurable disease;
c. no radiation therapy or chemotherapy within the last 4 weeks;
d. an age limit of 70 years;
e. a white blood cell (wbc) count greater than 5000 cells/mm³ and a platelet count greater than 100,000 platelets/mm³; and
f. no evidence of severe protein deficiency, infection, renal impairment, or severe liver function impairment.

A prestudy evaluation of the patient included:

a. hemoglobin level, wbc count, differential count, platelet count;
b. biochemical determinations of alkaline phosphatase, serum glutamic oxaloacetic transaminase, lactic dehydrogenase, bilirubin, creatinine, calcium, total protein, albumin;
c. chest roentgenogram; and
d. as indicated, liver scan, skeletal survey, etc.

During the course of study, hemoglobin level, wbc count, and biochemical determinations were carried out every 2 weeks.

Patients were treated orally with CCNU in a dosage of 130 mg/m² of body surface area, given as a single dose at 8-week intervals. Treatment was carried out until evidence of disease progression occurred and the patient was then taken off the study. Dose was modified as follows: if the wbc count was below 4000 but above 3000 cells/mm³, or if the platelet count was below 100,000 but above 75,000 platelets/mm³, 50% of the dose was given; if the wbc count was below 3000 cells/mm³ and the platelet count was below 75,000 platelets/mm³, the dose was not given and the patient was seen at 2-week intervals until the counts rose to acceptable levels. A minimum of one treatment course was considered to constitute an adequate trial. Tumor sites are shown in table 1. The patient population is shown in table 2.

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[1] Received Oct 17, 1973; revised Feb 22, 1974; accepted Mar 21, 1974.

[2] CCNU: CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-. Supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, National Cancer Institute.

[3] Chemotherapy Department, Ontario Cancer Foundation, and the Ottawa Civic Hospital, Department of Medicine, University of Ottawa, Canada.

[4] *Reprint requests to*: Dr. David J. Klaassen, The Ontario Cancer Foundation, Ottawa Clinic, Civic Hospital Division, Ottawa Civic Hospital, Ottawa, Canada K1Y 4E9.

[5] 5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU.

TABLE 1.—Tumor site

Site	No. of patients
Colon	21
Rectum	7
Stomach	7
Pancreas	3
Esophagus (adenocarcinoma)	1
Primary site unknown*	3
Hepatoma	1

*Metastatic adenocarcinoma histologically compatible with a gastrointestinal primary tumor; no other primary site was identified.

RESULTS

The criteria used to evaluate response are seen in figure 1. The response to therapy was disappointing, as outlined in table 3. One patient who had a complete response lasting for over 20 months had biopsy-proven liver metastases from a previously removed bowel carcinoma. One patient with adenocarcinoma of the esophagus had a disappearance of the palpable left supraclavicular lymphadenopathy for 3 months, but this was not accompanied by improvement in the patient's general condition. One patient had transient regression of metastatic supraclavicular lymphadenopathy secondary to carcinoma of the stomach, but had minimal subjective improvement. Three of the four good or complete responses were in previously untreated patients. Responses (if they occurred) were usually evident after the first course of the drug. Thus, there were only four good responses among 43 patients treated (9%). The remainder of the patients showed disease progression and were taken off the study.

TABLE 2.—Patient population

	Men	Women	Previously treated*	Previously untreated
No. of patients	21	22	32	11

*28 of 32 had previously received 5-FU alone or in combination; five of 32 had previously received methotrexate (NSC-740); 11 of 32 had previously received radiation therapy.

TABLE 3.—Response to CCNU therapy

Response	No. of patients	Site	Duration of response (mos)
Complete	3	Colon	20+
		Stomach	4
		Esophagus	3
Good-partial	1	Colon	12
Fair-partial	3	Bowel	12
		Carcinoid	3
		Hepatoma	2
Overall objective response	7/43* (16%)		
Overall complete and good-partial response	4/43* (9%)		

*No. of patients responding/total No. of patients tested.

a. Complete response--clearing of all lesions for a period of at least 2 months.

b. Good-partial response--50% tumor shrinkage for at least 2 months.

c. Fair-partial response--25%-50% shrinkage of tumor.

d. No response--no change in tumor parameters.

e. Progression--increase in tumor size.

f. Subjective response--no evidence of change in tumor parameters but the patient has symptomatic palliation, eg, pain relief, improvement in appetite, weight gain, or improvement in performance status.

FIGURE 1.—Criteria for responses.

Toxic effects are shown in table 4. Leukopenia tended to occur late, with the nadir being about 6–7 weeks after the drug was given. Thrombocytopenia tended to occur earlier than leukopenia at about the fourth to fifth week. Both the thrombocytopenia and leukopenia had a duration of approximately 2 weeks, and in most cases we were able to repeat the CCNU at the 8-week level. However, in the majority of cases the dosage schedule had to be reduced. A rather striking feature was the tendency of these patients to become anemic. Transfusions were required to support the hemoglobin level in most of the patients who had significant anemia. It was the general impression that the hematologic toxicity was more severe with the second or third course, although this was certainly not consistent. There were no drug-related deaths.

DISCUSSION

The results reported in this paper are consistent with results seen in other studies (6,7). Our response rate in previously untreated patients is significant and is consistent with the observations of other investigators. Many patients received CCNU late in the course of their disease. Nineteen patients received only one course of CCNU. Those patients who responded were in better general condition initially and were able to tolerate repeated courses of the drug. The degree of leukopenia and thrombocytopenia would probably have been more severe if a greater number of patients had been given more courses; many patients died or had disease progression after only one dose. All patients had adequate bone marrow reserve before CCNU was started. None had had previous extensive radiation therapy or chemotherapy.

TABLE 4.—Toxic effects of CCNU therapy

Toxic effects	No. of patients
Leukopenia (<4000 cells/mm³)	20
Thrombocytopenia (100,000 platelets/mm³)	9
Anemia (drop of hemoglobin level by 2 g/100 ml)	16
Nausea and vomiting	14

CCNU does show activity in gastrointestinal malignancies. Studies now in progress by the Eastern Cooperative Oncology Group are evaluating the effectiveness of a more active compound, methyl-CCNU,[6] alone and in combination with other agents in previously untreated gastrointestinal malignancies.

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[6] Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

Comparison of Daunorubicin (NSC-83142) With Adriamycin (NSC-123127) in the Treatment of Late-Stage Childhood Solid Tumors [1,2,3]

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SUMMARY

A randomized clinical study was designed to compare the complete and good partial response rates of daunorubicin and adriamycin in the treatment of children with various solid tumors. All children had received previous treatment. Both drugs were given for 5 consecutive days every 28 days. At the time of failure or no response, the alternate drug was started (part II). Analysis of 27 adequately treated patients in each group showed the response rate to be 15% after treatment with daunorubicin and 19% with adriamycin. Nineteen patients continued in part II and received the alternate regimen; no responses were seen. No difference was found in the response rate of patients with neuroblastoma. Of the nine patients with soft tissue sarcomas who were treated with adriamycin three had complete or good partial responses as compared to the one patient of five who had a good partial response after treatment with daunorubicin. If two additional objective responses of patients with soft tissue sarcomas were included (one mixed and one that lasted less than 28 days), there were five of nine patients who responded to adriamycin. No comparison could be made of response rates in Ewing's sarcoma, osteogenic sarcoma, lymphoma, Wilms' tumor, or teratoma. Hematologic toxicity was seen after the administration of both drugs but was slightly more marked in the daunorubicin regimen. Three children in each regimen received total doses that are sometimes associated with cardiac toxicity although it was not encountered.

[Cancer Chemother Rep 58:671–676, 1974]

Daunorubicin and adriamycin are antibiotics that were isolated from cultures of a mutant *Streptomyces peucetius caesius* by investigators in the Farmitalia Research Laboratories (1,2). These anthracycline compounds have a similar

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[2] Supported in part by Public Health Service grants CA-11796, CA-13809, CA-10382, CA-03888, and CA-07431 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3] Daunorubicin: CAS reg. No. 2341-50-6; daunomycin (NSC-82151). Daunorubicin and adriamycin were supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI.

[4] All authors are members of the Children's Cancer Study Group.

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chemical structure. Adriamycin differs from daunorubicin by the substitution of a hydrogen atom with a hydroxyl group on the acetyl radical. Since the isolation of daunorubicin early in 1960, there have been numerous reports of its effectiveness in the treatment of acute leukemia and a wide spectrum of solid tumors in children (3,4). In 1967, preclinical studies in experimental animals showed that adriamycin apparently had a higher therapeutic ratio than daunorubicin (2). Clinical studies reported between 30% and 50% objective responses in children with neuroblastoma, soft tissue sarcoma, Wilms' tumor, and lymphoma (5–8). Initially, adriamycin was considered to be less toxic, particularly to the myocardium, but more recently, myocardial toxicity has been recorded. It was difficult to determine from clinical reports of studies conducted during different time periods which of the two antibiotics was the most effective in the treatment of tumors, though published reports favored adriamycin. Thus, the investigators believed a randomized clinical trial was needed. The study herein reported was designed to: (*a*) compare the objective response rates of daunorubicin and adriamycin in various pediatric solid tumors, and (*b*) determine if cross resistance existed between the two antibiotics.

MATERIALS AND METHODS

Preliminary data suggested that the response rate of patients with pediatric solid tumors was about 20% for daunorubicin (3,4) and 40% or greater for adriamycin (5,7). The study was designed to evaluate a difference between 20% and 50% with 30 patients in each of two groups. It was hoped that enough patients with neuroblastoma would be entered in the study to allow a separate evaluation of this tumor; all other tumor types were to be considered as one. All patients had to have failed prior chemotherapy and had to be ineligible for phase III studies. Also required was a measurable index lesion not treated by radiation therapy. Six institutions in the Children's Cancer Study Group (CCSG) participated in the study.[*]

Patients were randomized into one of two regimens: (*a*) adriamycin (15 mg/m²/day × 5) given intravenously (iv), courses repeated every 28 days for 6 months; or (*b*) daunorubicin (30 mg/m²/day × 5) given iv, courses repeated every 28 days for 6 months. The dose levels and schedules selected were based on the report by Tan et al (7) which suggested that adriamycin produced comparable toxicity at half the dose (6).

For those patients who responded and remained in remission after 6 months, each agent was continued at the same dose level as a single injection every 2 weeks for 6 months, after which treatment was discontinued.

Patients with progressive disease after the first 5-day course and those with no regression of disease after two courses were changed to the alternate drug to form part II of the study. The dose regimen was the same as in part I. The following observations were required at the time of entry in the study: physical examination; complete blood cell count including platelet count or estimation and white blood cell count (wbc) differential; bone marrow aspiration; chest roentgenograms plus X-ray studies to document the extent of disease; serum glutamic oxaloacetic transaminase (SGOT) and blood urea nitrogen (BUN) values; EKG; and quantitative estimation of urinary vanillyl mandelic acid (VMA) for patients with neuroblastoma. Directly measurable lesions were recorded on CCSG tumor forms and flow sheets.

Followup observations were scheduled to include monthly physical examination and estimation of response: wbc on Days 0, 2, 4, and 14 of each course; bone marrow aspiration performed monthly if originally positive for tumor and at any time that unexplained leukopenia occurred; positive roentgenograms repeated monthly; BUN and SGOT determinations every 2 months; EKG every 3 months; and urinary VMA monthly if originally elevated in patients with neuroblastoma.

In the protocol, an adequate trial was defined as one 5-day course of treatment followed by

[*] The Children's Hospital of Philadelphia; Indiana University Medical Center; The Children's Orthopedic Hospital, Seattle; The National Children's Hospital, Washington, DC; Wyler Children's Hospital of the University of Chicago; and Children's Memorial Hospital, Chicago.

progressive disease or two courses in which there was no regression. Since some responses occurred during the first 2 weeks of treatment, all patients who survived 14 days or longer from initiation of chemotherapy are included in the analysis. The study was designed to analyze complete response (CR) and good partial response (GPR) only. A CR was defined as the disappearance of all evidence of disease for 28 days or more; a GPR was defined as a greater than 50% decrease in size of all measurable lesions for 28 days or longer.

Table 1 shows the randomization of patients, the number of evaluable patients, and the responses observed.

RESULTS

Between June 1971, and March 1973, 61 patients were entered in the study. Twenty-nine patients initially received daunorubicin and 32 initially received adriamycin. Between November 1972, and February 1973, all patients received daunorubicin due to an interruption in the supply of adriamycin. There were seven inadequate trials; six patients died during the first 2 weeks of study and the seventh patient was taken off study at the request of the parents after only one course of treatment with adriamycin. This child, with an abdominal neuroblastoma and massive involvement of the liver, received concomitant irradiation. Treatment led to a GPR that lasted several months. Nineteen patients continued in part II of the study and received the alternate agent; no responses were observed.

Four of 27 evaluable patients (15%) treated with daunorubicin had GPRs (table 1). The median duration of response was 115 days with a range of 56–160 days (table 2). The median time in the study was 31 days. There were one CR and four GPRs in 27 patients who received adriamycin (19%). The median duration of response was 120 days (range, 60–226 days) and the median time in the study was 63 days. All responses to both agents were initiated by the first course of treatment.

The response rate and duration for patients with neuroblastoma were the same for both drugs: two GPRs among 15 patients treated with daunorubicin and two GPRs among 13 patients treated with adriamycin. One of five patients with soft tissue sarcoma, a fibrosarcoma, receiving daunorubicin had a GPR which lasted 6 months. In the group that received adriamycin there were three responses in nine patients who had rhabdomyosarcoma. One of these responses was a CR that lasted 6 months in a patient with a rhabdomyosarcoma of the maxillary sinus. There was one GPR that lasted 5 months in a patient with metastatic Wilms' tumor who received daunorubicin. No chemotherapy-induced CRs or GPRs were observed in patients with Ewing's sarcoma, osteogenic sarcoma, lymphoma, or teratoma.

TABLE 1.—Tumor histology and responses to daunorubicin and adriamycin

	Daunorubicin		Adriamycin	
Disease	Total No. of patients	Total responses	Total No. of patients	Total responses
Neuroblastoma	15	2 GPR*	13	2 GPR
Rhabdomyosarcoma	4	0	9	1 CR, 2 GPR
Fibrosarcoma	1	1 GPR	0	
Ewing's sarcoma	0		3	(1 CR) †
Osteogenic sarcoma	3	0	0	
Lymphoma	3	0	0	
Wilms' tumor	1	1 GPR	1	0
Teratoma	0		1	0
Total	27	4 (15%)	27	5 (19%)

*GPR = good partial response with a greater than 50% regression of disease for more than 28 days.

†All lesions in the lungs received concurrent radiotherapy; therefore, this patient is not included in the analysis as a responder.

TABLE 2.—Results

	Daunorubicin	Adriamycin
Total No. of patients entered	29	32
Inadequate trials	2	5
Total No. of evaluable patients	27	27
Responses		
CR	0	1
GPR	4	4
Other objective*		4
None	4	12
Progressive disease	19	6
Time in the study (days)		
Mean	52	90
Range	14–196	22–282
Median	31	63
Duration of response (days)		
Median	115	120
Range	56–160	60–226

*Four patients receiving adriamycin achieved objective responses that lasted less than 28 days or the index lesions received concurrent radiotherapy.

The study was designed to analyze only CR and GPR in patients with measurable index lesions not treated with irradiation. There were four additional patients who had objective regression of disease after treatment with adriamycin who did not fit these criteria. One complete regression of pulmonary metastases from Ewing's sarcoma lasted 6 months, but all lesions received concomitant radiotherapy. Two patients (one with rhabdomyosarcoma and one with teratoma) had GPRs of less than 28 days' duration. An additional patient with an undifferentiated sarcoma had a mixed response that lasted 2 months with complete regression in some areas but growth in others.

TOXICITY

Table 3 summarizes the incidence of toxicity. A definition of the degree of hematopoietic depression is included in the table. Patients were rated according to the most severe level of toxicity that occurred during the time they were in the study. The hematologic toxicity was a little more marked after treatment with daunorubicin; 11 of 29 patients receiving daunorubicin had grade 4 as compared with eight of 29 receiving adriamycin. The effect was reflected most often by a depressed platelet count, 11 patients receiving daunorubicin having platelet counts of less than 24,000 cells/mm^3. One patient died of gastrointestinal bleeding during the early recovery phase of thrombocytopenia where the lowest platelet count was 5000 cells/mm^3.[7]

The majority of patients were able to keep on schedule and received the next course of treatment between 28 and 35 days, though the dose or number of injections was sometimes reduced. Table 4 gives details of the modifications and delays in the schedule. Other toxic effects seen consisted of two episodes (one on each drug) of fever related, by the investigator, to the drug, and one fatal seizure after the fifth dose of the first course of daunorubicin. This patient had lymphosarcoma with evidence of central nervous system (CNS) disease when entered

[7] Leukopenia grade 4 (wbc = 300 cells/mm^3) was seen in four of 29 patients who received daunorubicin and in 11 of 29 who received adriamycin. In patients with neoplastic infiltration of the marrow, leukopenia and thrombocytopenia were seen during the initial course; it is difficult to assess if the depression was primarily due to therapy or to a compromised marrow.

TABLE 3.—Incidence and definition of toxicity*

Drug	Total No. of patients	No. of patients with hematologic toxicity grade—					No. of patients with gastrointestinal toxicity grade—				
		0	1	2	3	4	0	1	2	3	4
Daunorubicin	29	0	4	12	2	11	24	0	5	0	0
Adriamycin	29	7	6	3	5	8	22	3	3	1	0

*Definition of toxicity:

	Grade			
	1	2	3	4
Hematopoietic toxicity				
Wbc (cells × 10^3)	2.4–1.5	1.4–0.75	0.74–0.3	0.29–0
Platelets (cells × 10^3)	149–100	99–50	49–25	24–0
Hemoglobin (g/100 ml)	9.9–8	7.9–6.5	6.4–5.0	4.9–0
Gastrointestinal toxicity	Nausea, mild stomatitis	Severe stomatitis	Diarrhea	—

in the study. The principal investigator considered the seizure as probably being related to disease rather than to medication, and the patient was considered an inadequate trial. Three patients received between 600 and 750 mg/m² of daunorubicin and three received between 450 and 525 mg/m² of adriamycin but no cardiac toxicity was noted. No obvious increase in infection was noted, although one patient who received an inadequate trial died on Day 13 with gram-negative sepsis during a drug-related period of leukopenia.

DISCUSSION

This study was designed to determine the objective response rate of daunorubicin and adriamycin in the treatment of childhood solid tumors. At the time of the study planning, it was felt that adriamycin was probably the superior drug. The study was constructed to analyze only CR and GPR and to detect a difference in response rate of 50% with adriamycin as compared to 20% with daunorubicin; these rates appeared to be the best estimates from the literature. The study failed to demonstrate any significant difference in the response rate, and the overall responses to both drugs are lower than previously reported. This may be explained in part by the type of patient entered in the study. The majority of these patients had advanced disease and perhaps did not survive long enough to benefit from the drug. Death was the reason for terminating treatment in one third of the patients. A median time in the study of 31 days in the evaluable patients receiving daunorubicin shows the status of patients entered. The time in the study was different for the two regimens: 31 days for daunorubicin and 63 days for adriamycin. This could be due to the superiority of adriamycin, but may also be the result of investigator bias. Since there was an initial belief that adriamycin would prove to be the better agent, there may have been a greater tendency to persist with adriamycin, or to be impatient with daunorubicin, leading to earlier rerandomization when daunorubicin was the initial agent. More patients received adriamycin when this was the second drug (13 compared to six).

The analyzed CR and GPR rates were the same after both drugs were given: 15% versus 19%. If the four additional objective regressions after treatment with adriamycin are included, the response rate is 33%. No difference was seen in children with neuroblastoma, but on examination of the results in patients with soft tissue sarcoma, we determined that adriamycin may be superior for this tumor: five of nine responses as compared with one of five. No conclusions can be drawn from this study regarding response rates in Ewing's sarcoma, osteogenic sarcoma, lymphoma, Wilms' tumor, or teratoma.

TABLE 4.—Modifications in dose and schedule

Courses	Total No. of courses	No. of courses at % of prescribed dose				Total No. of courses reduced/ % courses reduced	Total No. of courses postponed
		100%	75%	60%	40%		
Daunorubicin	49	38	5	2	4	11/22%	
Excluding initial course	20	18	0	0	2	2/10%	2
Adriamycin	72	63	4	4	1	9/12%	
Excluding initial course	44	37	2	4	1	7/15%	8

There was significant toxicity after both drugs, although it was slightly more marked in the daunorubicin regimen. This may be due in part to a larger number of patients who had tumor infiltration of the bone marrow in this group. It is always difficult to separate the toxic effect of the drug from that relating to marrow replacement. Gastrointestinal toxicity was not a problem; no cardiac toxicity was noted, but only three children in each group reached levels at which it might have been expected.

No responses were seen in patients receiving the alternate drug. The number of patients who crossed over was too few (13 and six) to allow any assessment of cross resistance for these two antibiotics.

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Comparison of 5-Azacytidine (NSC-102816) With CCNU (NSC-79037) in the Treatment of Patients With Breast Cancer and Evaluation of the Subsequent Use of Cyclophosphamide (NSC-26271)[1,2,3]

Thomas J. Cunningham,[4] Takuma Nemoto,[5] Dutzu Rosner,[5] Edward Knight,[6] Samuel Taylor,[7] Charles Rosenbaum,[6] John Horton,[6] and Thomas Dao [5,8]

SUMMARY

Fifty-eight patients with metastatic cancer of the breast were randomly allocated to receive 130 mg/m² of CCNU orally every 6 weeks or 60 mg/m² of 5-azacytidine intravenously daily for 10 days and twice weekly thereafter. Patients who failed to respond were treated on one of two dose schedules of cyclophosphamide. CCNU was well tolerated with the major toxic effect being delayed hematologic suppression. 5-Azacytidine produced intense gastrointestinal toxicity that often prevented maintenance therapy. There were few responses to either drug and only two patients responded to treatment with cyclophosphamide. Immunologic skin testing did not correlate with the extent of disease or the length of survival but the few responders had strong delayed hypersensitivity reactions.

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[3] 5-Azacytidine: CAS reg. No. 320-67-2; *s*-triazin-2-(1*H*)-one, 4-amino-1-β-D-ribofuranosyl-.

CCNU: CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-.

Cyclophosphamide: CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Drugs used in this study were supplied by Cancer Therapy Evaluation, DCT, NCI.

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Chemotherapeutic palliation is needed at some time in almost all patients with metastatic cancer of the breast (1). 5-Fluorouracil (5-FU) [9] and cyclophosphamide have been the most effective single agents, inducing remissions in approximately 25% of the patients (2, 3).

This cooperative study between the Albany Medical College (AMC) and the Roswell Park Memorial Institute (RPMI) was undertaken as a part of the plan of the DCT, NCI, in an effort to seek more effective single agents for the treatment of breast cancer.

5-Azacytidine and CCNU were the drugs chosen for trial. Weiss et al (4), using 5-azacytidine in a schedule similar to the one employed in this study, had noted seven of 11 objective responses and minimal toxicity in patients with breast cancer limited to soft tissues. CCNU had been shown to be effective in lymphomas (5) and glioblastomas (6) with

[9] 5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8.

some usefulness in gastrointestinal tumors (7) but had not received adequate trial in breast cancer. Patients whose disease progressed after having received one of these drugs were eligible for treatment with the "standard" agent, cyclophosphamide.

METHOD

Patients with measurable metastatic breast cancer no longer amenable to standard surgical and radiotherapeutic techniques who had not previously received these drugs were admitted to the study after submitting informed written consent. Patients were evaluated by history, physical examination, serum chemical profile, and chest and skeletal radiographs. Patients were stratified on the basis of predominant site of disease, tumor-free interval, and menopausal status (table 1) and were randomized to their treatment schedule by the closed-envelope technique.

The majority of evaluated patients were more than 1 year postmenopausal and had had a tumor-free interval, after initial treatment, of over 1 year (table 1). Patients with dominant osseous and visceral disease were equally represented and those with only soft tissue disease made up 22% of the entered patients. Six patients on each schedule had received prior cytotoxic chemotherapy with a single drug other than those used in this study. Eight patients from AMC and 18 from RPMI had had prior adrenalectomies and 28% of these patients had responded to the procedure.

The primary treatment phase compared the effectiveness of 5-azacytidine with that of CCNU. When disease progression occurred the patients were treated with one of two dose schedules of cyclophosphamide. 5-Azacytidine was given at a dose level of 60 mg/m² (not to exceed 100 mg) as an intravenous (iv) bolus each day for 10 days. A maintenance dose of 100 mg/m² (not to exceed 150 mg) was given twice weekly starting after the amelioration of hematologic toxicity from the initial course. CCNU was given at a dose level of 130 mg/m² orally and the dose was repeated every 6 weeks if the white blood cell count (wbc) was over 4000 cells/mm³ and the platelet count was over 75,000 platelets/mm³. The dose was delayed 2 weeks for patients with counts below these levels.

In the secondary treatment phase cyclophosphamide was given in one of two schedules: schedule A was 1 g/m² iv repeated every 3 weeks, and schedule B was 400 mg/m² iv daily for 4 days followed by a maintenance dose of 50 mg/day given orally. The dose was halved if the wbc fell between 5000 and 3000 cells/mm³ and was interrupted if the wbc fell below 3000 cells/mm³.

A complete remission was defined as a disappearance of all measurable disease. A partial remission was indicated if there was at least a 50% reduction in the sum of the product of the perpendicular diameters of all measured lesions in the absence of progressive disease. Progressive disease was defined as the occurrence of new disease or an increase in measured lesions to at least 125% of their original size. A treatment of at least 8 weeks was required before determining that the tumor was unresponsive.

The majority of patients were evaluated for response to skin testing before treatment. At

TABLE 1.—Stratification of patients with measurable metastatic cancer of the breast

Menstrual category	Total No. of patients	Dominant disease *					
		Visceral		Osseous		Soft tissue	
		<1 yr TF	>1 yr TF	<1 yr TF	>1 yr TF	<1 yr TF	>1 yr TF
<1 Yr postmenopausal	11	2	3	1	2	1	2
>1 Yr postmenopausal	43	8	8	6	12	2	7
Totals	54	10	11	7	14	3	9
		21		21		12	

*TF = the tumor-free interval from time of primary diagnosis until first recurrence.

AMC, 24 patients had a 2000-μg sensitizing dose and a 100-μg challenge dose of 2,4-dinitrochlorobenzene (DNCB) [10] which were simultaneously placed on the arm. The reaction to the challenge dose was interpreted at 14 days. If no reaction occurred, a repeat challenge dose of 100 μg was placed at the same site and read at 48 hours. A "mild" reaction was recorded if erythema or minimal induration was present in the area of the challenge application and a "strong" reaction was recorded if marked induration or blistering was noted. Twenty-four patients from RPMI were skin tested with the biologic agents intermediate purified protein derivative, Candida albicans (1:100 dilution), mumps antigen, and Varidase (10 units of streptokinase). The diameter of the induration at each site at 48 hours was scored as follows: 0 = 0–4 mm, 1 = 5–14 mm, 2 = 15–24 mm, 3 = 25–34 mm, 4 = 35 + mm. A mathematical sum of each skin test was used as the skin test score for each patient.

RESULTS

Fifty-eight patients were treated, 28 from AMC and 30 from RPMI. Fifty-four patients were evaluable for tumor response. In four patients the initial 10-day course of 5-azacytidine was interrupted because of severe gastrointestinal toxicity which prevented an adequate drug trial for antitumor effect. Equal numbers of patients were entered on each drug schedule in the primary treatment phase. Nineteen patients subsequently received cyclophosphamide on schedule A and 16 on schedule B.

Toxicity

Severe nausea and vomiting occurred in most of the patients who received 5-azacytidine, and in four patients it was so intolerable that the initial course of drug was not completed. Transient nausea and vomiting occurred in many patients shortly after taking the CCNU capsules. Hematologic toxicity was frequent but of moderate severity in patients treated with 5-azacytidine, whereas thrombocytopenia and leukopenia frequently delayed subsequent courses of CCNU (table 2). There were no drug-associated deaths. One patient treated with 5-azacytidine died 10 days after the initial course of therapy. A postmortem examination revealed myocardial and perivascular edema but showed no necrosis or fibrosis. The cause of death was probably due to hepatic failure from metastasis. Generalized weakness developed in one patient treated with CCNU and intellectual function deteriorated. No metastasis or other cause could be determined by brain scan, electroencephalography, or physical examination. Progression of disease during treatment was the cause of death in 12 patients who received 5-azacytidine and in nine patients who received CCNU. Hematologic toxicity was of moderate intensity in both regimens of cyclophosphamide but occurred more frequently in the high-dose intermittent schedule.

Response

Two of 27 patients who received 5-azacytidine

[*] 2,4-Dinitrochlorobenzene: NSC-6292; CAS reg. No. 97-00-7; benzene, 1-chloro-2,4-dinitro-.

TABLE 2.—Toxicity

Toxic effects	No. of patients receiving—*			
	5-Azacytidine (31)	CCNU (27)	Cyclophosphamide†	
			A (19)	*B* (16)
Wbc <3000 cells/mm³	14	14	9	3
Platelets <100,000 platelets/mm³	3	17	2	3
Gastrointestinal toxic effects	28	19	13	6
Alopecia	1	—	13	8

*The total No. of patients treated with each drug is in parentheses.

†*A* = 1 g/m² q3wks. *B* = 400 mg/m² given iv for 4 days followed by oral therapy.

and three of 27 who received CCNU had partial remissions. There were no complete remissions. The mean duration of response was 5.5 months for both groups. Only one of the 14 patients with disease limited to soft tissue had a remission. None of the 12 patients who had received prior chemotherapy responded. The mean length of survival was 11.8 months for responders and 6.1 months for nonresponders. Four patients receiving 5-azacytidine and six receiving CCNU had stable disease for variable periods but progression eventually occurred.

A partial remission occurred in two patients treated with cyclophosphamide on schedule B. Three patients on each cyclophosphamide schedule had stable disease and the remaining patients had disease progression.

Skin Testing

Seven of the 24 tested patients entered from AMC had positive DNCB skin tests. The only strong reactions of the seven positive skin tests occurred in three of eight patients who had soft tissue disease only. The remaining four positive reactions were very mild and occurred both in patients with visceral and osseous dominant disease. Only one of four tested patients from RPMI with disease limited to soft tissue had a high skin test score. Five of the 12 patients who received prior chemotherapy had positive DNCB skin tests. The only responder at AMC had a strongly positive DNCB reaction and all three of the responders from RPMI that were skin tested had high skin test scores. The length of survival was not significantly different in patients who responded differently to DNCB or biologic skin testing.

DISCUSSION

The study design allowed some comparison to be made between the effectiveness and toxicity of the two primary agents tested in patients with advanced breast cancer as well as allowing a determination of the effectiveness of the two schedules of cyclophosphamide given as secondary therapy. In this fashion, too, patients were not denied treatment with a "standard" agent.

We were unable to confirm the optimistic report of Weiss et al (4) on the effectiveness of this dose schedule of 5-azacytidine in the treatment of breast cancer. The difference in response rate cannot readily be explained by differences in patient populations since more patients with disease limited to soft tissues were treated in the present study and fewer patients had received prior cytotoxic therapy than those reported by Weiss. Gastrointestinal toxicity from this drug was so severe that the majority of patients had difficulty completing their initial course. This characteristic of 5-azacytidine, combined with its low index of activity, severely limits its potential usefulness in the management of breast cancer.

CCNU was also relatively ineffective in our hands although it was tolerated much better than 5-azacytidine. Activity has been demonstrated in some other tumors (5–7) and Hoogstraten et al (8) reported seven of 36 partial responses in patients with breast cancer. The hematologic toxicity of CCNU on this dose schedule was of moderate intensity. Neurologic toxicity from CCNU was suggested in one patient but the pathogenesis was not determined.

Cyclophosphamide, used as a secondary treatment drug, was also ineffective when compared to the expected 28% remission rate when this drug is given as the first chemotherapy to similar patients (3). The patients in this study entering the cyclophosphamide phase had a poor clinical performance status after previous chemotherapy. Our low rate of remission may have resulted from exposure to ineffective agents which rendered the cancer unresponsive to a known effective agent. Talley et al (9) have shown that 38% of the patients who responded to treatment with either 5-FU or cyclophosphamide had another response when they were treated with the alternate drug, whereas few patients responded to the crossover drug after the initial failure to respond. A similar effect may follow previous chemotherapy with 5-azacytidine and CCNU.

Evaluation of the skin test data did not produce correlations with differences in disease extent or length of survival except that only those patients with disease limited to soft tissue had strong reactions to DNCB. The therapeutic response rate was too small to correlate defi-

nitely with skin testing, yet all of the responders had strong skin test reactions. Further application of skin test evaluation is currently in progress.

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Drug Trials in Neuroblastoma: Cyclophosphamide (NSC-26271) Alone; Vincristine (NSC-67574) Plus Cyclophosphamide; 6-Mercaptopurine (NSC-755) Plus 6-Methylmercaptopurine Riboside (NSC-40774); and Cytosine Arabinoside (NSC-63878) Alone [1,2]

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SUMMARY

Twenty-three children with generalized neuroblastoma who had not received prior therapy were treated with either cyclophosphamide alone given in pulse fashion, or with cyclophosphamide and vincristine given on alternate weeks. One of 12 children treated with cyclophosphamide alone achieved a complete response, seven had partial responses, two had mixed responses, and two did not respond. Two of 11 children treated with vincristine and cyclophosphamide achieved complete responses, three achieved partial responses, and six did not respond. Twelve children whose disease was resistant to cyclophosphamide and vincristine were treated with 6-mercaptopurine and 6-methylmercaptopurine riboside and none responded. Eleven children whose disease was resistant to cyclophosphamide and vincristine were treated with cytosine arabinoside and none responded.

[Cancer Chemother Rep 58:683–688, 1974]

The addition of chemotherapeutic agents to surgical treatment and irradiation therapy has done little to change survival and cure rates in children with neuroblastoma over the past 15 years (1–3), but it has been helpful in the palliation of generalized disease and in the prolongation of survival (4–8). Over a 3-year period the Pediatric Division of the Southwest Oncology Group studied four drug regimens in the treatment of patients with metastatic neuroblastoma.

METHODS

Children with a tissue diagnosis of neuro-

[1] Received Jan 14, 1974; revised Apr 11, 1974; accepted Apr 22, 1974.

[2] Compounds used in this study were supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, National Cancer Institute (NCI). Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

[3] This study was done by the Southwest Oncology Group. The following institutions participated in the study: Baylor College of Medicine, Houston, Tex (*Kenneth A. Starling*; CA-03161 and CA-07357); University of Texas M. D. Anderson Hospital and Tumor Institute, Houston (*Wataru W. Sutow*; CA-03713); University of Virginia School of Medicine, Charlottesville (*Milton H. Donaldson*; CA-08223; presently at the Children's Hospital, Philadelphia, Pa); Washington University School of Medicine, St. Louis, Mo (*Vita J. Land*; CA-05587); University of Oklahoma Health Sciences Center, Oklahoma City (*Daniel M. Lane*; CA-11233; presently at Oklahoma City Clinic); University of Arkansas Medical Center, Little Rock (CA-03400); Tulane University School of Medicine, New Orleans, La (CA-03389); Henry Ford Hospital, Detroit, Mich (CA-04915); Cleveland Clinic, Ohio (CA-04919). The Southwest Oncology Group is supported by grants CA-03754, CA-12014, and CA-12644. All grants are from the NCI, National Institutes of Health, Department of Health, Education, and Welfare.

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blastoma were eligible for these studies if they had generalized disease that could not be definitively treated surgically or with irradiation therapy. Measurable lesions were required for entry into the study.

Complete response was defined as the disappearance of all measurable lesions and any subjective complaints. Partial response was defined as a 50% reduction of all measurable lesions; mixed response was defined as a 50% reduction in one or more measurable lesions with progression of other new or old lesions. No response was defined as less than 50% reduction or no reduction in the size of any measurable lesions.

The four drug trials were divided into three studies. A phase III study compared "pulse" cyclophosphamide with cyclophosphamide and vincristine given alternately at weekly intervals. Children who had not received prior treatment with cyclophosphamide or vincristine were randomized to one of the two treatment programs: (*a*) Cyclophosphamide was given at a dose level of 250 mg/m²/day orally until the white blood cell count (wbc) reached a nadir of 1000 cells/mm³. If the nadir was not reached in 14 days, the dose was increased to 300 mg/m²/day, then to 350 mg/m²/day, etc, at 2-week intervals until the nadir was reached. A rest period of 6 weeks with no therapy followed and subsequently another pulse was given. Therapy was continued until there was evidence of progressive disease. (*b*) Vincristine (1.5 mg/m²) was administered intravenously (iv) every 2 weeks alternating at weekly intervals with cyclophosphamide (300 mg/m²) given orally or iv every 2 weeks. A course of therapy for each of these regimens was at least 12 weeks.

The second study, for the treatment of children whose disease was resistant to vincristine and cyclophosphamide, utilized a combination of 6-mercaptopurine (6-MP), 300 mg/m²/day, and 6-methylmercaptopurine riboside (6-MMPR), 100 mg/m²/day, for 5 days. This was followed by a 2-week rest period and the drugs were reinstituted on Day 19. Three courses of treatment were considered an adequate trial.

The third study was also utilized for patients whose disease was resistant to vincristine and cyclophosphamide therapy. Cytosine arabinoside was given at a dose level of 100 mg/m²/day by rapid iv drip for 5 days followed by a 2-week rest period. Subsequent courses were given at the same interval and dose level. If the children had received over 3000 rads or intensive chemotherapy within the past 6 weeks, 50 mg/m²/day was given for 5 days followed by a 2-week rest period. If toxicity was tolerable, the dose was escalated to 75 mg/m²/day on the same schedule. Two courses were required for an adequate trial.

RESULTS

Vincristine-cyclophosphamide versus "pulse" cyclophosphamide.—Thirteen children were randomized to receive vincristine and cyclophosphamide alternately (table 1A). One patient who was treated for only 3 weeks was not included for evaluation. One of the remaining 12 children was not eligible for the study because he had localized disease and no measurable lesion. He achieved complete remission and is disease free more than 2 years after therapy was discontinued. Eleven children were evaluated for response to this therapy. Six children who were treated from 6 weeks to 5 months had no objective response. All had progressive disease at the time this drug regimen was discontinued. Three children achieved partial remissions but had progressive disease at the end of 3 months and therapy was changed. One child achieved complete remission and is disease free more than 4 years after therapy was stopped. Another child achieved complete remission but progressive disease developed during continued therapy. Toxic effects are listed in table 1A and are similar to those previously reported for these drugs. Fourteen children were randomized to receive "pulse" cyclophosphamide (table 1B). Two patients were not included for analysis: one because of major protocol violation (drug was given at improper dose level) and the other because of early death on Day 14. One of the remaining 12 patients achieved a complete response of 8 months' duration. Seven achieved partial responses and two had mixed responses. Only

TABLE 1A.—Vincristine and cyclophosphamide

Patient No.	Age at diagnosis	Age at therapy	Involvement	Duration of therapy	Response*	Toxicity
1	8/12	9/12	Lungs, pleura	2 yrs	CR and cure	Leukopenia, moderate
2	9/12	9/12	Abdomen, liver, spleen, bone	2 yrs	CR, then progression	None
3	1 7/12	1 9/12	Multiple bone, nodes	3 mos	PR, then progression	Marrow depression, moderate
4	6 5/12	6 5/12	Soft tissue, bone marrow	3 mos	PR, then progression	Constipation, moderate; nausea, moderate
5	1 4/12	1 4/12	Multiple bone	3 mos	PR, then progression	None
6	4 3/12	4 3/12	Thorax, bone marrow	5 mos	NR	None
7	1 2/12	1 2/12	Liver, abdomen	6 wks	NR	None
8	2 5/12	2 5/12	Bone marrow, bone, abdomen	3 mos	NR	None
9	3 1/12	3 1/12	Bone marrow, abdomen	2 mos	NR	None
10	4/12	4/12	Soft tissue	3 mos	NR	None
11	1 4/12	1 4/12	Abdomen, bone marrow, bone	3 mos	NR	Leukopenia, moderate

*CR = complete response, PR = partial response, NR = no response.

two patients did not respond to therapy. The principal toxicity observed was neutropenia.

6-MP–6-MMPR.—Thirteen children were treated with 6-MP and 6-MMPR. One child received only one dose of the drugs and died on the first treatment day. Three children received only one course of the drug; four children received two courses of the drug. None of these children responded and all had progressive disease. Five children received three or more courses of the drug combination. None responded and all had progressive disease. The toxicity observed in this study was similar to that reported previously for these two drugs and the toxic effects are listed in table 2.

Cytosine arabinoside.—Eleven children received cytosine arabinoside. Only one child was treated at the 100-mg/m² dose level. The remainder received 50 mg/m²/day for 5 days. All but two of these patients received only one course. One of these two patients received a second course at the 75-mg/m²/day level for 5 days and the other patient received three courses at the 50-mg/m² level. Seven of the 11 children died during the first treatment period (19 days). All of these deaths were due to progressive disease and not to the toxic effects of the drug. The remaining four children were treated for 19, 36, 46, and 54 days, respectively. None showed any response and all had progressive disease. Three patients had moderate bone marrow suppression.

DISCUSSION

Table 2 summarizes the results of the three studies. The phase II trials with cytosine arabinoside and 6-MP–6-MMPR were performed because each of these drugs was shown to be effective in the treatment of acute leukemia. In the past, other drugs with antileukemic activity also have exhibited an antitumor effect in neuroblastoma. No responses were seen in children who received the two regimens. These studies were done during the time that dose regimens for cytosine arabinoside were being determined and it is possible that other sched-

TABLE 1B.—Pulse cyclophosphamide

Patient No.	Age at diagnosis	Age at therapy	Involvement	Duration of therapy	Response*	Toxicity
1	1 4/12	1 5/12	Bone marrow, nodes	4 courses (9 mos)	CR, 8-mo duration	Leukopenia, severe
2	4 9/12	4 10/12	Abdomen, nodes, bone marrow, bone	3 courses (8 mos)	PR, then PD	Leukopenia, severe
3	4 8/12	4 8/12	Bone marrow, multiple abdomen	3 courses (7 mos)	PR, then PD	Leukopenia, moderate
4	2 7/12	2 8/12	Nodes, muscle, bone, bone marrow	4 courses (6 mos)	MR, then PD	Nausea + vomiting, moderate; hematuria, severe; bone marrow, severe
5	3 4/12	3 4/12	Bone, bone marrow, abdomen, liver, spleen	3 courses (4 mos)	PR, then PD	None
6	2 8/12	2 9/12	Abdomen, bone	2 courses (4 mos)	PR, then PD	Frequency, mild; leukopenia, severe; anemia, moderate; nausea + vomiting, moderate
7	1 4/12	1 4/12	Abdomen, lung	1 course (3 mos)	MR, then PD	None
8	1 7/12	1 7/12	Liver, soft tissue	1 course (1 mo)	PR, then PD	Leukopenia, severe; nausea + vomiting, moderate
9	10 1/12	10 2/12	Multiple bone	3 courses (5 mos)	PR, then PD	Nausea + vomiting, mild; leukopenia, severe
10	2 7/12	2 7/12	Abdomen, nodes, bone	3 courses (2 mos)	PR, then PD	None
11	5 9/12	5 9/12	Abdomen, bone	3 courses (4 mos)	NR	Neutropenia, severe; anemia, moderate; dysuria, moderate
12	4 6/12	4 7/12	Bone marrow, abdomen	3 courses (5 mos)	NR	Leukopenia, severe; anemia, severe

*CR = complete response, PR = partial response, MR = mixed response, PD = progressive disease, NR = no response.

TABLE 2.—Summary of responses

	Complete response	Partial response	Mixed response	No response	Total patients
Pulse cyclophosphamide	1	7	2	2	12
Vincristine + cyclophosphamide	2*	3	0	6	11
6-MP + 6-MMPR	0	0	0	12	12
Cytosine arabinoside	0	0	0	11	11

*One cure, no evidence of disease 4 yrs after therapy was stopped.

TABLE 3.—Comparison of vincristine-cyclophosphamide studies

Study (reference No.)	Complete response	Partial response	Mixed response	No response	Total patients
Pulse cyclophosphamide (present study)	1	7	2	2	12
Cyclophosphamide + vincristine (present study)	2	3	0	6	11
Vincristine (8)	1	2	—	6	9
Vincristine (8)	1	1	—	7	9
Vincristine + cyclophosphamide (6)	2	6	—	13	21
Vincristine + cyclophosphamide (6)	5	5	—	10	20
Vincristine + cyclophosphamide (6)	7	9	—	12	28
Vincristine + cyclophosphamide (9)	4	5	—	19	28

ules may have some effect against neuroblastoma.

Cyclophosphamide and vincristine have shown the greatest antitumor effect in neuroblastoma. There was no statistically significant difference between the response rates of the two arms of the phase III study, although more patients responded in the group that received cyclophosphamide alone (66% complete + partial responses vs 45% complete + partial responses with the two-drug combination). There was little toxicity in the vincristine-cyclophosphamide arm. The moderate to severe neutropenia noted in nine of 12 children who received cyclophosphamide alone was expected since a total wbc of 1000 cells/mm^3 was the end point of drug administration.

Table 3 lists the response rates of neuroblastoma to cyclophosphamide and vincristine in three recent studies (6,8,9). In these studies a greater percentage of children under 1 year of age were included. Since children under 1 year of age have a better prognosis, even with generalized disease, the response rates in those studies are not comparable to those reported here.

In summary, occasional good responses can be obtained with the use of drug therapy in children over 1 year of age, but the overall response rate has not been impressive. For the most part, responses have been only partial in degree and transient in duration.

LIST OF COMPOUNDS

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride

6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate; 6-MP

6-Methylmercaptopurine riboside: NSC-40774; CAS reg. No. 7143-67-1; 9*H*-purine, 6-(methylthio)9-β-D-ribofuranosyl-, dihydrate; 6-MMPR

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Effectiveness of Methyl-GAG (NSC-32946) Administered Intramuscularly[1,2,3]

Bruce I. Shnider,[4] Jacob Colsky,[5] Ralph Jones,[6] and Paul P. Carbone[7,8]

SUMMARY

Methyl-GAG has been reported to be an active agent when given intravenously in the therapy of acute myelocytic leukemia. This study was undertaken to evaluate the effectiveness of this agent administered intramuscularly to patients with solid tumors. The results indicate the feasibility of using the intramuscular route of administration for studies of maintenance therapy or for the study of the action of this chemotherapeutic drug in solid tumors. Although not a specific objective of the initial study, responses were observed in carcinoma of the breast, esophagus, oral cavity, rectum, and in malignant lymphoma.

[Cancer Chemother Rep 58:689–695, 1974]

In 1958, a study by Freedlander and French demonstrated the antitumor activity of a newly synthesized chemotherapeutic agent, methyl-GAG, in mouse L1210 leukemia (1). Mihich and his co-workers undertook extensive antitumor and preclinical pharmacologic studies with this agent (2,3). Their studies demonstrated the undesirable side effects of this new agent to be gastrointestinal toxic reactions, bone marrow depression, and severe hypoglycemia. These toxic effects were found not only in animals, but in man as well. Although the animal data indicated that the therapeutic effectiveness of this agent was similar when given by the oral and parenteral routes, human studies clearly demonstrated the lack of antitumor activity when methyl-GAG was administered orally (4). After parenteral dosage (two thirds of the radioactive intravenous [iv] injection of a single dose) in the mouse with tagged methyl-GAG, only 60% was excreted in the urine and less than 20% in the stool after 3 weeks (5). The recovered excretion product was intact drug in both species. Clinical studies (6) revealed that this agent has activity in acute myelocytic leukemia and in malignant lymphomas. Therapeutic effectiveness required the iv administration of the drug and was associated with undesirable effects such as laryngitis, pharyngitis, colitis,

[1] Received Jan 14, 1974; revised May 7, 1974; accepted May 23, 1974.

[2] This study was performed by the Eastern Cooperative Oncology Group (Bruce I. Shnider, chairman; Paul P. Carbone, current chairman) and the former Eastern Drug Evaluation Program (George E. Moore, chairman) and was supported in part by Public Health Service grants CA-02824 and CA-02822 from the National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health, Education, and Welfare.

[3] Methyl-GAG: CAS reg. No. 24968-67-0; guanidine, 1,1'-[(methylethanediylidene)dinitrilo]di-, dihydrochloride, monohydrate; methylglyoxal-Bis-guanylhydrazone. Supplied by Cancer Therapy Evaluation, Division of Cancer Treatment (DCT), NCI.

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conjunctivitis, laryngeal ulcerations, hypoglycemia, and bone marrow depression (7).

Studies were undertaken to determine the feasibility of administering methyl-GAG by other than the iv route in view of the reports of its significant therapeutic activity in acute myelocytic leukemia (8–10) and the desirability of developing a technique for maintenance therapy for this and other malignant diseases. Methyl-GAG was administered subcutaneously to patients at an initial dose of 1 mg/kg and increased to 4 mg/kg. At the latter dose, local toxicity produced a severe reaction at the injection site that was associated with local hemorrhage, ulceration, and slough. A second patient received intramuscular (im) methyl-GAG with minimal evidence of local toxicity. The dose was progressively increased and after only four daily doses of 6 mg/kg, laryngitis, diarrhea, and leukopenia occurred. This suggested that the tolerated dose of im methyl-GAG might be less than that of the iv route. It also indicated that deep im injections might be a feasible form of maintenance therapy. Subsequent to these preliminary findings, the Eastern Cooperative Oncology Group (ECOG) and the Eastern Drug Evaluation Program (EDEP) undertook a phase I–II protocol study of im administration of methyl-GAG to patients with solid tumors.

METHOD

Concentrations of 0.1 and 0.2 mg/ml of methyl-GAG were prepared by the Clinical Center Pharmacy Department of NIH. The drug was supplied as a lyophilized powder in sterile ampules suitable for parenteral administration. Prior to administration, the contents of the ampules were dissolved in 10 ml of sterile distilled water so that each milliliter of solution contained 100 mg of this drug. The drug was administered by the deep im route in the upper outer quadrant of the buttocks twice a week at an initial dose of 3 mg/kg. Modification of the dose (increase or decrease) was to be determined by the investigators after evaluating any toxic effects that occurred. Patients were carefully observed at twice weekly intervals in regard to their hematologic status, blood chemistries, chest roentgenograms, objective and subjective reactions, and body weight. Whenever possible, weekly measurement and enumeration of tumor masses were performed by all investigators. Objective response was defined as a 50% or more regression of tumor masses that persisted for at least 2 consecutive weeks. Since this study was designed primarily for evaluation of drug dose schedule and route of administration, no attempt was made to study survival time or prolonged duration of effect beyond the period of drug administration.

RESULTS

Sixty patients were studied in the initial drug evaluation which was conducted by the ECOG, and 68 patients were studied in the evaluation conducted by the EDEP. It was found that 3–4 mg/kg of methyl-GAG given im could be tolerated by patients on a twice weekly schedule (6–8 mg/kg total weekly dose) without severe toxic reactions. Tables 1 and 2 demonstrate the results of the ECOG study and the side effects encountered. The most striking toxic effects consisted of myelosuppression, anorexia, nausea, and vomiting.

In a few instances, patients experienced a moderately severe burning sensation that began circumorally and then involved the face and spread over the entire body at the time of, or a few minutes after, the injection. This sensation was very brief, lasting only a few seconds to minutes. Sometimes a patient experienced dizziness, weakness, and faintness 5–15 minutes after the injection when assuming the upright position. Postural hypotension, however, could not be documented clinically when these symptoms occurred.

In the EDEP study, moderately severe anorexia was the most commonly encountered undesirable toxic effect of methyl-GAG treatment. This occurred usually during the second or third week of treatment and tended to persist until treatment was discontinued. Weight loss was associated with the anorexia. Vomiting and diarrhea occurred in some patients, but were not severe enough to discontinue therapy. On occasion, ulcerative stomatitis, proctitis, and colitis were seen. When larger doses of the drug were given, tracheitis,

TABLE 1.—ECOG phase I study of methyl-GAG—weekly schedule

Dose (mg/kg)	No. of patients	No. of weeks on drug	Total dose (g)	Toxic effects (No. of patients)				
				Blood cell counts				
				Wbc (<3000 cells/mm³)	Platelets (<100,000 platelets/mm³)	Anorexia and nausea	Vomiting	Circumoral tingling and facial flush
6	4	8–17	3.0–6.25	1	0	0	0	—
8	3	5–9	1.3–4.4	0	1	4	2	1
10	6	5–12	3.7–6.7	1	1	3	3	2
12	3	8–10	5.9–9.7	2	2	3	2	1
15	2	7	3.8–5.1	2	2	2	2	2
Total No. of patients	18							

TABLE 2.—ECOG phase I study of methyl-GAG—twice weekly schedule

Dose (mg/kg)	No. of patients	No. of weeks on drug	Total dose (g)	Toxic effects (No. of patients)						
				Blood cell counts						
				Wbc (<3000 cells/mm³)	Platelets (<100,000 platelets/mm³)	Anorexia and nausea	Vomiting	Stomatitis	Circumoral tingling	Dizziness
3	2	8–20	3.5–5.1	0	1	0	0	1	0	0
4	18	6–9	3.5–5.0	3	2		3	4	5	0
6	8	4–6	3.4–5.6	3	3	6	4	6	5	1
8	6	3–4	2.6–4.6	5	4	6	4	5	6	1
Total No. of patients	34									

bronchitis, and occasional pneumonitis were encountered. Muscle aching and weakness involving the large proximal muscles of the arms and legs occurred without marked objective findings.

Inflammatory skin lesions resembling erythema multiforme were sometimes seen. This skin reaction had a tendency to be generally distributed and occurred late in the course of drug administration. It subsided rapidly when the drug was discontinued. Only three patients in this series manifested this side effect.

Although investigators were informed of the need to be sure that the drug was given by deep im injection, there were a few instances when it was inadvertently given by the subcutaneous route. When this occurred it became evident within 24 hours; a severe local induration developed at the injection site associated with pain and tenderness. This was followed in 24–48 hours by localized fat necrosis, by localized hemorrhage, and on two occasions by a small area (1.5–2 cm) of subcutaneous and cutaneous ulceration (this was resolved spontaneously within a few weeks leaving a pigmented spot which gradually faded). As there was no serious morbidity associated with this complication, drug administration could be continued while the patient was recovering from these toxic reactions.

Five objective responses were seen in the 55 patients participating in the ECOG study and 11 objective responses were seen in the 68 patients participating in the EDEP study (tables 3–5). Responses were not necessarily associated with toxicity, but a somewhat higher rate of response did occur in the group having some form of toxic effects from the drug (table 6) as compared with the nontoxic group.

The objective responses were usually of short duration (4–8 weeks), but one patient with lymphosarcoma did have a partial response which lasted 10 months before relapse occurred.

CONCLUSIONS

This experience indicates that methyl-GAG has some activity in human solid tumors. The

TABLE 3.—ECOG phase I study—responses

Patient No.	Disease	Sex	Dose schedule (mg/kg)	Toxic effects	Comments
1	Lymphosarcoma	F	(3.0) twice weekly for 20 wks	Sore throat initially and alopecia that subsided during therapy	Regression (50%) for 10 mos; then progression of disease
2	Carcinoma of esophagus	F	(4.0) twice weekly for 4 wks	Subcutaneous nodules at injection site; dizziness and sweating after injection	Partial (50%) decrease in metastatic chest nodule for 8 wks after second injection
3	Breast	F	(6.0) twice weekly for 6 wks	Nausea, diarrhea, and constipation; perirectal irritation	Measured lesions decreased in size but number of lesions increased
4	Reticulum-cell sarcoma	M	(6.0) twice weekly for 6 wks	Leukopenia and severe nausea and vomiting	2 remissions both occurring after 2 wks of therapy
5	Carcinoma of esophagus	M	(6.0) twice weekly for 8 wks	Wbc to 3400 cells/mm³	50% decrease in pulmonary nodule for 4 wks; then progression

TABLE 4.—ECOG phase I study: patient response to methyl-GAG by disease

Disease	No. of patients	No. of partial responses	No. of subjective improvements
Carcinomas			
Breast	6	2	3
Colon	6	0	0
Lung	8	0	2
Rectum	3	0	1
Esophagus	3	2	1
Ovary	2	0	0
Cervix	4	0	2
Kidney	2	0	2
Parotid	2	0	0
Stomach	1	0	0
Tongue	1	0	0
Larynx	2	0	0
Uterus	1	0	0
Undetermined origin	2	0	0
Sarcomas			
Lymphosarcoma	3	2	2
Reticulum-cell sarcoma	2	0	0
Osteogenic sarcoma	1	0	0
Fibrosarcoma	1	0	0
Lymphomas			
Hodgkin's	5	0	1
Mycosis fungoides	3	1	1
Others			
Sacral chordoma	1	0	0
Astrocytoma	1	0	0
Totals	60	7	13

TABLE 5.—EDEP phase II study: responses to methyl-GAG by primary tumor site

Tumor site	No. of patients	No. of complete responses	No. of partial responses
Connective tissue	9	0	2
Large intestine	9	0	2
Mouth, pharynx, larynx	7	0	2
Kidney	5	0	1
Undetermined	5	0	0
Lung	4	0	0
Salivary gland	3	0	0
Stomach	3	0	1
Breast	2	0	0
Lymphoma	2	0	2
Melanoma	2	0	0
Rectum	2	1	0
Skin	2	0	0
Testis	2	0	0
Bladder	1	0	0

TABLE 5.—EDEP phase II study: responses to methyl-GAG by primary tumor site—Continued

Tumor site	No. of patients	No. of complete responses	No. of partial responses
Central nervous system	1	0	0
Cervix	1	0	0
Esophagus	1	0	0
Lip	1	0	0
Liver-bile passage	1	0	0
Miscellaneous	1	0	0
Ovary	1	0	0
Prostate	1	0	0
Tongue	1	0	0
Uterus	1	0	0
Totals	68	1	10

TABLE 6.—EDEP phase II study: relationship of toxicity or nontoxicity to response

Patients	No. of patients with toxic effects	No. of patients without toxic effects	Total
Responders	8	3	11
Nonresponders	35	22	57
Totals	43	25	68

undesirable toxic effects encountered with prolonged administration may make it difficult to administer the compound for an extended period of time. The numbers of patients in any specific tumor group in this study were small, but tumor responses were observed in patients with carcinoma of the breast, esophagus, oral cavity, and rectum. Four partial responses were also seen in patients with malignant lymphomas. In some patients, response was noted without undue host toxicity. The feasibility of administering methyl-GAG by the im route has been demonstrated. The observed results suggest that selected solid tumors are responsive to this im route and that further exploration of the effectiveness of methyl-GAG given im may be of value for those tumors responding to the agent.

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Intermittent High-Dose Cyclophosphamide (NSC-26271) Treatment of Stage III Ovarian Carcinoma[1,2,3]

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SUMMARY

Nine patients with stage III ovarian carcinoma were treated with multiple courses of cyclophosphamide (120 mg/kg). Eight of the nine patients responded to therapy with a greater than 50% reduction in tumor mass and seven had complete disappearance of palpable tumor. Two patients had no detectable tumor as shown by laparoscopy and cytology following treatment. The duration of response varied from 2 to greater than 40 months with a median of 10 months. The median survival from the start of therapy was 18 months (8–>40). One patient has survived free of disease for over 40 months. Patients had severe pancytopenia with white blood cell and platelet nadirs of 28–90 cells/mm^3 and 15,000–35,000 platelets/mm^3 respectively. Myeloid marrow recovery was prompt, and there was no increased leukopenia or delayed leukocyte recovery after repetitive doses. However, most patients had progressive delay in platelet recovery, resulting in dosage modifications in two patients. High-dose cyclophosphamide is extremely effective in reducing tumor masses in patients with ovarian carcinoma but its advantage over conventional intermittent melphalan therapy has yet to be demonstrated.

[Cancer Chemother Rep 58:697–703, 1974]

Alkylating agents produce responses in 45%–75% of patients with epithelial ovarian carcinomas (1). Melphalan[6] given intermittently has been the drug most extensively studied, resulting in responses in approximately 50% of the patients treated (2,3). Cyclophosphamide is also effective and produces responses in 75% of patients (4). The best alkylating agent and the most effective schedule of administration for treatment of ovarian carcinoma have yet to be determined. Therapy with other classes

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[3] Cyclophosphamide: CAS reg. No. 6055-19-2; 2*H*-1,3,-2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino] tetrahydro-, 2-oxide, monohydrate. Supplied by Mead Johnson & Co., Evansville, Ind.

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[6] Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-.

of drugs has been less effective and for practical purposes alkylating agents are the only agents which produce significant and lasting tumor regressions (1–3). Recent studies with hexamethylmelamine [7] suggest activity for this agent but extensive clinical trials have not been performed (5).

Bruce et al have defined cyclophosphamide as a class III, cycle-specific agent with relatively less effect on resting stem cells than on proliferative lymphoma cells (6). Stem cells are drawn from the resting phase into the proliferative state to repair marrow injury (7,8). These data provide the rationale for the administration of maximally tolerated doses of cyclophosphamide repeated as soon as hematologic recovery has occurred and marrow stem cells have returned to the resting phase (9). This concept was proven to be valid in the clinical studies of Bergsagel et al (10). Previous studies from our institution in patients with a variety of malignancies have shown that patients tolerate high doses of cyclophosphamide (120 mg/kg) with rapid marrow recovery despite profound pancytopenia (11). In addition, it was found that the reinfusion of stored autologous marrow did not hasten the already rapid marrow recovery (12). Several patients with ovarian carcinoma treated with high-dose cyclophosphamide showed dramatic tumor regressions. The purpose of this study was to determine the efficacy and toxicity of repeated high doses of cyclophosphamide in patients with ovarian carcinoma.

[7] Hexamethylmelamine: NSC-13875; CAS reg. No. 645-05-6.

METHODS

Patients

Nine patients with stage III (extension of tumor beyond the true pelvis but not outside the abdominal cavity) ovarian carcinoma were included in this study. Table 1 presents the clinical status of these patients at the start of therapy. Five patients (Nos. 1, 2, 4, 5, and 7) had biopsy or limited surgery with large tumor masses remaining; two patients (Nos. 3 and 9) had extensive surgery with relatively small but palpable implants remaining at the time of therapy; and one patient (No. 6) had no palpable tumor. Patient No. 8 had moderate tumor masses that recurred 20 months after prior surgery for stage I disease. No patient had received prior chemotherapy. Patient No. 7 had received 300 rads of radiation to the pelvis prior to this study.

Plan of Treatment

The basic plan of treatment was to administer 60 mg/kg (2.2 g/m^2) of cyclophosphamide on each of 2 successive days for a total dose of 120 mg/kg (4.4 g/m^2) every 4–6 weeks for a minimum of six cycles, followed by a

TABLE 1.—Clinical status of patients

Patient No.	Histologic type	Age (yrs)	Ascites?	Surgery	Residual tumor*
1	Serous	42	Yes	Biopsy	4+
2	Serous	48	No	Oophorectomy	4+
3	Undifferentiated	42	No	BSO † and hysterectomy	2+
4	Serous	51	No	Oophorectomy	4+
5	Undifferentiated	50	Yes	Biopsy	4+
6	Serous	40	No	BSO and hysterectomy	1+
7	Undifferentiated	58	No	Biopsy	3+
8	Serous	36	No	BSO and hysterectomy	2+
9	Undifferentiated	37	No	BSO and hysterectomy	2+

* 1+ = Minimal tumor implants, not palpable; 2+ = minimal but palpable tumor; 3+ = moderate palpable tumor; 4+ = large unresected tumor.

† BSO = bilateral salpingo-oophorectomy.

second-look procedure by laparotomy or laparoscopy with pelvic washings for cytologic examination. Patients were then to receive maintenance chemotherapy and/or pelvic and abdominal strip irradiation.

Management of Patients

At first, patients were hospitalized from the time of drug administration until recovery of peripheral blood cell counts. As experience was obtained patients were discharged after receiving cyclophosphamide and readmitted only for infectious complications during the period of pancytopenia. Platelets were administered prophylactically when the platelet count fell below 20,000 platelets/mm^3.

Drug Administration

Cyclophosphamide (60 mg/kg, 2.2 g/m^2) was dissolved in 250 ml of D5W and infused intravenously (iv) in 30–60 minutes, usually in the evening. Patients were given Compazine and phenobarbital prior to cyclophosphamide to ameliorate nausea and vomiting. Iv fluids (6–8 liters/day) in conjunction with diuretics (furosemide) were administered to maintain copious diuresis in order to prevent cystitis.

Evaluation

Complete blood cell counts were performed at least three times per week until marrow recovery. Electrolyte, creatine phosphokinase, serum glutamic oxaloacetic transaminase, blood urea nitrogen, and alkaline phosphatase determinations were performed at least weekly. Periodic physical and pelvic examinations were performed to ascertain clinical response. Partial response was defined as an estimated reduction in tumor volume of greater than 50% and a clearing of all ascites. A complete response was defined as the disappearance of all palpable tumor masses. Laparoscopy or laparotomy with cytologic examination of saline washes was performed in six patients to further evaluate response.

RESULTS

General Toxicity and Dosage Modifications

Two patients (Nos. 1 and 6) completed the prescribed six courses of cyclophosphamide at full doses. Patient No. 5 received eight courses of cyclophosphamide because she had residual tumor after her sixth course. Patient No. 8 relapsed prior to the sixth course and was removed from the study. Patient No. 2 received courses of 60, 90, and 100 mg/kg after ischemic EKG changes occurred following the first course of cyclophosphamide, but she received the last two doses at 120 mg/kg without further cardiac problems. Patient No. 3 was given only 60 mg/kg in her first course due to a lack of wound healing from surgery. She had severe and prolonged cystitis and her second course consisted of mechlorethamine [8] (1 mg/kg). She received four subsequent courses of cyclophosphamide (120 mg/kg) without further cystitis. Patient No. 4 received 60 mg/kg in the sixth course due to excessive delay in platelet recovery. Patient No. 7 received 10–30 mg/kg in courses 3–7 due to excessive thrombocytopenia and delayed platelet recovery. Patient No. 9 received only four courses so that we could administer irradiation after a complete response.

Antitumor Effect

Eight of nine patients responded to therapy, six completely and two partially (table 2). The single nonresponder did not have palpable tumor at the start of therapy but relapsed between the fifth and sixth courses of cyclophosphamide. Five of the six patients with complete clinical responses were further evaluated by laparoscopy or laparotomy and three of these had residual tumor. The remaining patient who achieved a complete response underwent surgery with bilateral salpingo-oophorectomy, hysterectomy, omentectomy, and resection of remaining visible tumor after the second course of cyclophosphamide; she refused a second-look procedure after the sixth course of the drug when no tumor was palpable.

The median survival from the start of therapy in the nine patients was 18 months (range, 8−>40). The nonresponding patient survived 8 months and the two patients with

[8] Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride; nitrogen mustard.

TABLE 2.—Treatment and response to cyclophosphamide

Patient No.	No. of courses	Total dose (mg/kg)	Treatment period* (mos)	Response		Post-cyclophosphamide therapy ‡	Duration of response (mos)	Survival (mos)
				Clinical	Lap †			
1§	6	720	11.0	CR	—	Melphalan	10	20
2	6	610	6.0	PR	Pos	Surgery and melphalan	14	23
3¶	5	540	6.0	CR	Neg	Melphalan and irradiation	>40	>40
4	6	660	7.0	CR	Pos	Cyclophosphamide maintenance	14	21
5	8	900	6.0	CR	Pos	—	2	13
6	6	720	5.5	NR	—	—	0	8
7	7	400	9.0	CR	Pos	Irradiation	9	16
8	5	600	5.5	PR	—	—	4	9
9	4	480	3.5	CR	Neg	Irradiation	9	18

* Time from first to last dose.

† Laparotomy or laparoscopy with cytologic examination. Pos = visible tumor or positive cytology. Neg = no visible tumor and negative cytology.

‡ Therapy after cyclophosphamide and prior to relapse.

§ Surgery (BSO and hysterectomy) after second course.

¶ Second course = 1 mg/kg of mechlorethamine substituted for cyclophosphamide because of cystitis.

partial responses survived 9 and 23 months. Patients with complete responses survived from 12 to more than 40 months.

The duration of response varied from 2 to more than 40 months with a median of 9–10 months. One patient is alive and free of disease more than 40 months after the start of therapy and has not been treated for over 12 months.

Three patients (Nos. 5, 6, and 8) relapsed during therapy. Four patients (Nos. 1, 2, 3, and 4) received maintenance therapy with melphalan or cyclophosphamide. Three patients (Nos. 3, 7, and 9) received irradiation to the pelvis and abdomen in an attempt to eradicate remaining tumor.

Hematologic Toxicity

Table 3 details the hematologic effects of multiple courses of cyclophosphamide (120 mg/kg). Lower doses were omitted from this analysis. The nadir of the white blood cell count (wbc) occurred on Day 11 (range, Days 9–13) with recovery to over 1.0×10^3 cells/mm^3 by Day 17 (range, Days 12–20). The wbc nadir and recovery time did not vary with repeated courses of cyclophosphamide. There was a tendency towards lower initial platelet counts with repeated courses and a slower recovery time of platelets. Two patients had dosage modifications due to platelet toxicity. Patient No. 4 received 60 mg/kg in the sixth course because of delayed platelet recovery following courses 4 and 5. Patient No. 7 had prolonged platelet recovery after course 2, became refractory to random platelet transfusions, and tolerated a maximum of only 30 mg/kg in subsequent courses. No patient in this series had clinical bleeding but platelets were administered prophylactically for counts of less than 20,000/mm^3.

Fever and Infectious Complications

Fever greater than 101 F occurred following 27 of 41 courses of cyclophosphamide at 120 mg/kg. The median duration of fever was 4 days (range, 1–13). Antibiotics were administered to patients with documented infection after 14 of 41 courses and after an additional eight courses with suspected but not documented infection. There were three episodes of

TABLE 3.—Hematologic toxicity from multiple courses of cyclophosphamide (120 mg/kg)

Course No.*	No. of patients per course	Wbc			Platelet count			No. of patients requiring platelet transfusion
			Day of —			Day of —		
		Initial/nadir (median/mm³)	Decline< 1000 cells	Rise> 1000 cells	Initial/nadir (median × 10³/mm³)	Decline< 100,000 cells	Rise> 100,000 cells	
1	8	7600/40	10	16	492/35	10(5)†	15	1
2	8	5800/40	10	15	598/31	12(7)†	15	4
3	7	6600/90	11	15	420/28	10(6)†	17	0
4	7	5000/50	10	15	323/15	10(6)†	19	4
5	7	7700/40	11	16	293/19	9(6)†	21	4
6	5	6900/28	11	16	252/32	9(4)†	21	2

* Median interval between courses = 35 days (range, 24–180).
† Nos. in parentheses = No. of patients with platelet counts <100,000.

septicemia, all associated with local infection in the pharynx or perianal area. Fever and infection cleared after antibiotic therapy and recovery of marrow function.

Nonmarrow Toxicity

Nausea and vomiting occurred in most patients after cyclophosphamide administration. Three patients had symptomatic cystitis on nine separate occasions. The only severe case followed an initial dose of 60 mg/kg and was associated with moderate hematuria and prolonged pain that persisted for 30 days. Mechlorethamine (1 mg/kg) was substituted for the second course. However, the patient tolerated four subsequent courses of cyclophosphamide (120 mg/kg) with minimal symptoms. All patients had total alopecia limited to the scalp. No significant renal or hepatic toxicity was seen. Mucositis was occasionally seen during the period of granulocytopenia. One patient had EKG changes of anterior wall myocardial ischemia 14 days after the first course of cyclophosphamide. She was given reduced doses of 60–100 mg/kg for the next three courses and full doses for the last two courses without further evidence of myocardial toxicity. There was no evidence of myocardial toxicity in the other patients.

DISCUSSION

These studies demonstrate that most patients tolerate intermittent large doses of cyclophosphamide. The predominant toxicity was hematologic with profound pancytopenia followed by rapid recovery of marrow function between courses. No cumulative toxicity was seen for myeloid marrow but repeated courses progressively lowered the platelet count and lengthened the platelet recovery time. Marrow toxicity was severe and infectious complications were documented or suspected in 34% of the patients. Dosage modification was necessary in two patients because of delayed platelet recovery. Nonmarrow toxicity was limited to nausea and vomiting, total alopecia, and cystitis.

Cumulative toxicity to the bladder, liver, heart, and other organs was not observed. This is of particular importance with respect to cardiac toxicity. In man, cardiac toxicity has been reported after single doses of 240 mg/kg (11,12) and suspected at doses of 200 mg/kg (13); in addition, such toxicity has been well documented in nonhuman primates after doses of 240 mg/kg (14). The absence of cardiac toxicity in our group of patients confirms our clinical impression that this specific toxicity is related to treatment where the total dose is given at once and does not occur with intermittent doses of 120 mg/kg or less.

Comparison of responses in this small group of patients with responses in other series in the literature is difficult. The "standard" therapy for disseminated ovarian carcinoma is melphalan given intermittently as described by Smith et al (2,3); such therapy produced responses in 47% of the patients (20% complete and 27% partial). The overall median survival was 12 months. The median survival was approximately 20 months for responders and less than 1 year for nonresponders. Length of response was related to the histologic type of disease, with mucinous tumors giving the longest, undifferentiated tumors the shortest, and serous tumors intermediate responses (3). In the present series a high response rate was seen (eight of nine patients) and the overall survival (median, 18 months) compares favorably to other series, especially since all of our patients' tumors were of the less favorable type. Seven of nine patients were alive at 1 year and one at 2 years. Intermittent melphalan therapy produced a 1-year survival rate of 51.5% and a 2-year survival rate of 17% (3).

The overall toxicity of these high doses of cyclophosphamide is greater than that seen with intermittent melphalan (2,3) with more morbidity and hospitalization time.

The number of patients treated in this study is too small for definite conclusions to be reached regarding the role of high-dose cyclophosphamide in the treatment of stage III ovarian cancer.

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Increased Clinically Symptomatic Pulmonary Radiation Reactions With Adjuvant Chemotherapy[1]

Kent B. Lamoureux [2,3]

Since excellent discussions and descriptions are available in the leading radiotherapy textbooks (1–3), the clinical, radiologic, and pathologic manifestations of radiation reactions of the lung should be well known to the radiotherapist. Unfortunately, the standard diagnostic textbooks pay little attention to these reactions (4,5). Currently, many investigators are utilizing combinations of radiotherapy and chemotherapy. In a randomized pilot study of lung cancer comparing radiotherapy and concurrent chemotherapy with radiotherapy alone, which I initiated in the Radiation Branch of the National Cancer Institute, it was my clinical impression that the patients receiving combined therapy had earlier and more marked radiographic changes after the course of radiotherapy and experienced much more severe constitutional symptoms of acute radiation pneumonitis as well. Therefore, analysis of the patients in the study for symptomatic and radiologic evidence of radiation reactions in the treated lung was undertaken.

MATERIALS AND METHODS

Patients referred to the Radiation Branch of the National Cancer Institute with a biopsy-proven diagnosis of lung carcinoma, unresectable by exploration or other surgical criteria but without gross clinical evidence of metastatic disease beyond the mediastinum, undergo a thorough workup to rule out metastatic disease. This workup includes multiple scanning and radiographic procedures, immunologic evaluations, and blood chemistry evaluations. In our original study those patients who were without evidence of metastases were randomized according to cell type to receive radiation therapy alone or radiation therapy plus concomitant chemotherapy.

Radiation therapy in both groups was given in identical fashion. Split-course radiotherapy, consisting of two sections of 2750 rads each, separated by a rest period of 23 days, was utilized in all patients except the patient reported in the case report (*see* Results). Opposing anterior and posterior portals were used, designed to include all known disease with a generous margin, and the mediastinum was included 3 cm across the midline. The 2750 rads were given in 14 or 15 equal fractions, treating both fields daily, Monday through Friday. Full chest tomography was employed to evaluate the margins of the disease.

Chemotherapy consisted of mechlorethamine, procarbazine, and vincristine given in 28-day cycles according to the schedule described by DeVita et al (6).[4] In all cases, the chemotherapy was started 3 days prior to the beginning of radiotherapy except in one patient who received his first drug 7 days after beginning radiotherapy.

RESULTS

There were 11 patients who demonstrated no evidence of disease beyond the mediastinum. All completed their total planned course of radiotherapy, and five were treated with concurrent chemotherapy.

[1] Received Jan 31, 1974; revised Apr 26, 1974; accepted May 23, 1974.

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[3] *Reprint requests to:* Kent B. Lamoureux, MD, Radiation Branch, Division of Cancer Biology and Diagnosis, National Cancer Institute, Bldg 10, Rm B3B38, Bethesda, Md 20014.

[4] Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride; nitrogen mustard.

Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-*α*-(2-methylhydrazino)-, monohydrochloride.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

Of the six patients treated with radiotherapy alone, only one had symptoms during or after completion of therapy indicative of possible radiation pneumonitis. These symptoms consisted of a body temperature of 101–102 F for 7–10 days, a nonproductive cough, and mild shortness of breath beginning 18 days after the final treatment. A followup chest roentgenogram taken 23 days after the onset of symptoms demonstrated moderate but definite changes indicative of radiation reaction. Two other patients had marked radiographic changes consistent with the treatment portal with later significant progressive fibrosis but were without symptoms consistent with acute pneumonitis. In addition, two patients had changes minimally indicative of fibrosis. The sixth patient had no evidence of fibrosis.

All five patients treated with combined therapy had moderate to severe symptoms of probable pulmonary origin during their therapy. Three had a symptom complex of fever with temperatures of 100–102 F for a few days, nonproductive cough, weakness and malaise, and minimal shortness of breath. Symptoms began 10–13 days after completion of radiation; chest roentgenograms were taken of all three patients during their febrile stage, showing no evidence of pneumonia or radiation changes and with continued tumor regression. All three, on their next followup chest roentgenogram, showed marked changes consistent with radiation reaction in the portal area. Two of these patients had normal white blood cell counts (wbc), but their chemotherapy was delayed due to the symptoms. The third patient had a wbc of 1900 cells/mm^3 and was hospitalized and treated for pneumonia. In a fourth patient, severe leukopenia and tumor obstruction with septicemia developed during the course of therapy; the patient subsequently recovered enough to resume therapy but died of uncontrolled liver metastases within 3 weeks of completing his course of radiation. The fifth patient is presented as a case report.

Case report.—Nine days after completing the first half of his planned split-course radiotherapy (2750 rads in 3 weeks) and concurrent combination chemotherapy, this patient (a 58-year-old man) was admitted to his local hospital by his referring physician with a temperature of up to 104 F, marked weakness, shortness of breath, and a wbc of 2770 cells/mm^3. After multiple cultures were taken, he was given intravenous antibiotics with a working diagnosis of lung abscess with septicemia. His temperature remained elevated for 2 weeks before it eventually subsided; no pathogenic organism was identified. When the patient was examined in our department 25 days after the onset of these severe symptoms (34 days after completion of the radiotherapy), a chest roentgenogram showed the treatment area completely involved with changes consistent with early radiation changes. A review of all previous roentgenograms showed no evidence of an abscess formation; in fact, a progressive decrease in tumor size had occurred. It was felt that his acute symptoms were due to an acute case of radiation pneumonitis.

DISCUSSION

The clinical symptoms and radiologic findings of radiation reactions of the lung have been well described elsewhere (1–3). In one series (7), 35 of 50 patients who were treated after mastectomy showed radiographic evidence of radiation fibrosis, and seven of these 35 had symptoms of pneumonitis, a pattern that our small group of six patients treated with radiotherapy alone seems to have followed. Some authors state that acute radiation pneumonitis usually develops 3–6 weeks after radiation therapy (1), while others found no consistent relationship in the time of onset of either symptoms or radiographic changes and no definite relationship between the symptoms and later radiographic changes (2). All seem to agree that symptomatic cases are rare, however, most reported experiences involve treatment with continuous radiation therapy alone and this "usual" latent period might not exist under split-course conditions, with or without concurrent chemotherapy. Symptoms developed in the single patient in the radiation-therapy-alone group 18 days after his final treatment, and symptoms of probable acute radiation pneumonitis developed in the four patients undergoing combination therapy within 14 days of their final radiation treatment.

The appearance of fever and respiratory symptoms with or without significant pulmonary infiltrates in a patient with carcinoma of the lung is usually first considered to be due to infection from an obstructing tumor. When interpreting the film the radiologist is often unaware of the details of previous therapy, and may be unaware of possible sequelae of that therapy, either from chemotherapy or radiation therapy (8). A diagnosis of infection may lead to a prolonged hospitalization and unnecessary treatment with high-potency antibiotics (as with the patient in the case report). Such a diagnosis may also prevent effective therapy to be instituted against radiation pneumonitis since many authors feel that steroids may be of help in the acute symptomatic stage (9), although probably not in the later stages (1,9). However, the use of antibiotics or steroids prophylactically in the absence of clinical indications is not generally recommended (1). In all five cases cited in this report, the first chest roentgenogram taken after subsidence of the symptoms showed significant changes compatible with radiation reaction, but in four of the patients, chest roentgenograms taken at the height of the symptoms showed no evidence of lung change other than further tumor regression.

Over a period of months in totally asymptomatic patients, the irradiated lung volume can undergo progressive changes that appear very ominous to a radiologist who is unfamiliar with the possible sequelae of radiation to the lung. An erroneous diagnosis of recurrent or metastatic tumor can lead to discarding an effective chemotherapeutic program, beginning unnecessary chemotherapy, or even considering unnecessary surgical treatment (7,8).

Review of the radiation field sizes in the symptomatic patients treated with combined chemotherapy and radiotherapy indicates the possibility that field size may influence the severity of the acute symptoms, since the patients with the most severe symptoms had the larger field sizes. This is an idea that will be pursued in our later studies. The clinical impression that the radiologic evidence of radiation fibrosis appears earlier also cannot be adequately evaluated with the present material.

Many drugs alone have been recognized as the cause of pulmonary complications with diffuse radiographic changes (10,11) and recently procarbazine has been added to this list (12).

The augmented local skin and mucous membrane responses to irradiation in patients treated with actinomycin D (NSC-3053) are well known (13), and the possible danger of treating lung tissue concurrently with these two modalities has been recognized (13,14). Considerable thought has been given to increased tumor response when chemotherapy is combined with radiotherapy, but often the other side of the coin, increased normal tissue reactions to combination therapy, is neglected (15). Recently a synergistic effect of irradiation and vincristine has been postulated as a cause of transverse myelitis (16). It is certainly logical to assume that other chemotherapy agents in combination with radiotherapy may bring out undesirable side effects and significant complications.

In conclusion, it would appear reasonable to warn those engaged in combination therapy involving radiation therapy and chemotherapy of the possibility of increased radiation reactions. With respect to radiation pneumonitis, this warning is not intended to discourage aggressive therapy. I would hope to prevent the institution of unnecessary or incorrect therapy and to prevent the possible discontinuation of effective chemotherapy, as well as to make investigators mindful of the possible increased complications when planning the course of combined therapy. Furthermore, it is my clinical impression that skin reaction to radiation is increased after numerous cycles of chemotherapy with the MOPP [5] program as described by DeVita et al (6), although this has yet to be evaluated completely. The above conclusions and the report by Byfield (15) on the possible synergistic effect of vincristine and radiation therapy leading to radiation myelitis at a much lower dose than would be expected, highly suggest that increased reactions be looked for in

[5] MOPP = mechlorethamine, vincristine (Oncovin), prednisone, and procarbazine.

Prednisone: NSC-10023, CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-.

all organ systems that are irradiated during combination studies. With respect to a patient showing symptoms of chest pathology, including fever, severe cough, dyspnea, the possibility of acute symptomatic radiation pneumonitis should be considered in the differential diagnosis if the patient has recently received radiotherapy to the chest, especially if it was given after or coincident with chemotherapy.

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High-Dose Cyclophosphamide (NSC-26271) for the Treatment of Metastatic Testicular Neoplasms [1,2,3]

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SUMMARY

Nine patients with disseminated germinal-cell testicular neoplasms (seminomas excluded) were treated with two to five courses of 60–120 mg/kg of cyclophosphamide. Eight of these nine patients responded to therapy with a greater than 50% reduction in tumor and two patients had a complete disappearance of detectable tumor. One patient who did not respond to two courses of 120 mg/kg of cyclophosphamide died in 3 months. Six patients with partial responses sustained remissions of 2–5 months' duration and died 5–20 months after the start of therapy. One patient with a complete response refused maintenance therapy, relapsed in 4 months, and died 19 months after therapy began. One patient who had spinal cord compression and widespread metastases is alive and free of disease at 24 months. He was given combination chemotherapy maintenance for 12 months. Patients receiving cyclophosphamide at a dose of 60 mg/kg had median white blood cell and platelet nadirs of 200 cells/mm^3 and 90,000 platelets/mm^3, respectively, and at a dose of 120 mg/kg median white blood cell and platelet nadirs were 30 cells/mm^3 and 19,000 platelets/mm^3, respectively. Infectious complications occurred after one of 12 courses of cyclophosphamide at 60 mg/kg and after 15 of 18 courses at 120 mg/kg. There were four episodes of septicemia in the latter group. Initially, high-dose cyclophosphamide was extremely effective in reducing tumor size in patients with testicular tumors but responses were usually of short duration.

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Metastatic germinal testicular neoplasms are tumors with a short doubling time (1). Their rapid growth probably explains the high initial response rate of 50%–70% to a variety of chemotherapeutic agents and combinations of agents (2–9). It is reported that 10%–30% of patients with these tumors

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[3] Cyclophosphamide was supplied by Mead Johnson, Evansville, Ind. Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

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achieve complete responses (2–9). One large series reported that 50% of the patients achieving complete responses survived 2 years or more (3). Despite the high response rate, however, the median survival time is less than 12 months (2–9).

Alkylating agents have been used to treat patients with disseminated seminomas (3,10) but have not been extensively evaluated in other testicular neoplasms, except in combination with other agents at relatively low doses (2–6,9). The animal studies of Bruce et al classified cyclophosphamide as a phase-specific agent that has relatively less effect on hematopoietic colony-forming cells than on proliferative lymphoma cells treated with increasing doses (11). Other studies have demonstrated that resting hematopoietic colony-forming cells are drawn into the proliferative phase after marrow injury but return to a resting state with marrow recovery (12,13). These data form the theoretical basis for the intermittent administration of high doses of cyclophosphamide as outlined by Bergsagel et al (14,15).

Studies from this institution previously demonstrated that doses of 60–120 mg/kg of cyclophosphamide were tolerated in man with rapid marrow recovery after a period of severe pancytopenia (16). Patients with ovarian carcinoma have received 120 mg/kg of this agent every 4–6 weeks without prohibitive cumulative toxicity (17). The purpose of this study was to evaluate the efficacy and toxicity of intermittent high-dose cyclophosphamide (60–120 mg/kg) in patients with metastatic testicular tumors.

METHODS

Patients

Nine patients with disseminated testicular neoplasms were included in this study. Table 1 presents the pertinent clinical data. All patients had disease metastatic above the abdomen (stage III) (including pulmonary metastasis) at the time of therapy except for patient No. 3 who had only retroperitoneal involvement (stage II). Eight of the nine patients were secreting human chorionic gonadotropic hormone (HCGH).

Treatment Plan

The original treatment plan was the administration of 60–120 mg/kg of cyclophosphamide given at 3–4-week intervals and for at least two courses beyond a complete re-

TABLE 1.—Pretreatment clinical status of patients

Patient No.	Age	Histology	HCGH*	Prior treatment†
1	27	Teratocarcinoma	Yes	O
2	47	Embryonal	Yes	O, ND, Rad
3	36	Teratocarcinoma	Yes	O, ND
4	33	Embryonal	No	O
5	22	Teratocarcinoma with embryonal	Yes	O, ND, Rad
6	31	Teratocarcinoma with embryonal	Yes	O
7	19	Teratocarcinoma with embryonal	Yes	O, Rad
8	23	Embryonal	Yes	O, ND, Rad, Chemo
9	25	Teratocarcinoma with embryonal	Yes	O

*HCGH = human chorionic gonadotropic hormone. Yes = elevated blood levels; No = normal levels.

†O = orchiectomy; ND = node dissection; Rad = radiation to retroperitoneal lymph nodes; Chemo = chemotherapy with sarcolysin and vinblastine.

sponse. Patients were given 60 mg/kg if they were thought to have poor bone marrow reserves (based on prior irradiation therapy or chemotherapy) and 120 mg/kg if they were thought to have good marrow reserves. Patients who achieved complete response status were to be given combination chemotherapy maintenance for 2 years. Irradiation therapy was administered for severe local problems in conjunction with chemotherapy.

Drug Administration

Cyclophosphamide (60 mg/kg, 2.2 g/m^2) was dissolved in 250 ml D5W and infused intravenously (iv) in 30–60 minutes, usually in the evening. Patients were given Compazine and phenobarbital prior to cyclophosphamide to ameliorate nausea and vomiting. Iv fluids (6–8 liters/day), in conjunction with diuretics (furosemide), were administered to maintain copious diuresis in order to prevent cystitis.

Evaluation

Complete blood cell counts were performed at least three times per week until marrow recovery was seen. Electrolyte, creatine phosphokinase, serum glutamic oxaloacetic transaminase, blood urea nitrogen, and alkaline phosphatase determinations were performed at least once per week. Periodic physical and roentgenographic examinations were performed to ascertain patients' clinical responses. Lymphangiography was repeated in patients with complete responses. HCGH production was evaluated prior to treatment and at least monthly thereafter. This was determined by direct radioimmunoassay of HCGH or indirectly by radioimmunoassay of human luteinizing hormone (HLH), where the cross-reaction between the tumor gonadotropin and HLH was utilized (18). A partial response was defined as an estimated reduction in tumor by greater than 50% for 1 month. A complete response was defined as a disappearance of all palpable and X-ray-visible tumors and a disappearance of HCGH production.

Management of Patients

Initially, patients were hospitalized from the time of drug administration until the recovery of their peripheral blood cell counts. As experience was obtained, patients were discharged after receiving cyclophosphamide and they were readmitted only for infectious complications during the period of pancytopenia. Platelets were administered prophylactically for platelet counts below 20,000 platelets/mm^3.

RESULTS

Antitumor Effects

The results of therapy are summarized in table 2. One patient (No. 1) who failed to respond had a 95% reduction in HCGH titer at the first course of cyclophosphamide with minimal changes in pulmonary metastasis. The second course of cyclophosphamide produced transient changes in pulmonary metastasis with rapid disease progression that was unresponsive to other chemotherapeutic agents.

Six patients achieved a partial response lasting 1–3 months but all relapsed while receiving cyclophosphamide. All were subsequently treated with multiple agents including actinomycin D. In most instances there were transient responses, but significant prolongation of survival occurred in only one patient (No. 7) who had successive partial responses to a variety of agents and survived for 20 months.

Two patients (Nos. 4 and 9) responded with a complete disappearance of tumor. Patient No. 4 refused further therapy and relapsed 4 months after the fifth course of cyclophosphamide. Patient No. 9 was admitted with widespread metastatic disease associated with an HLH titer of 28,800 mIU. He also had lower spinal cord compression with paraplegia. Two days after administration of the first course of cyclophosphamide, radiation therapy was instituted and over an 8-day period he received 2400 rads of ^{60}Co to the lower thoracic and lumbar areas with complete resolution of neurologic signs and symptoms. Because of severe marrow toxic effects associated with the first course of cyclophosphamide, he received 60 mg/kg on the second course. No tumor could be found after the third course of cyclophosphamide (120 mg/kg) and HLH titers were normal. He was given maintenance therapy with a combination of agents includ-

TABLE 2.—Treatment and response

Patient No.	Dose (mg/kg)	No. of courses	Total dose of cyclophosphamide (mg/kg)	Treatment period* (mos)	Response†	Duration of response (mos)	Survival (mos)
1	120	2	240	2	NR	—	3
2	120	1	300	3	PR	2	5
	60	3					
3	120	4	480	7	PR	5	13
4	120	5	600	8	CR	12	19
5	120	2	300	3	PR	2	8
	60	1					
6	120	2	240	2	PR	2	6
7	60	5	300	4	PR	3	20
8‡	60	2	220	2	PR	3	9
	100	1					
9§	120	2	300	4	CR	>20	>24
	60	1					

*Time from first to last dose.

†NR = no response; PR = partial response; CR = complete response.

‡Third course of cyclophosphamide given as 10 mg/kg on each of 10 successive days.

§ During first course of cyclophosphamide he received 2400 rads ^{60}Co irradiation to lower thoracic and lumbar areas. Maintained with combination chemotherapy for 1 year after CR.

ing actinomycin D, sarcolysin, bleomycin, mithramycin, vincristine, and 5-fluorouracil. Maintenance therapy was discontinued after 12 months and he has been free of disease for over 2 years since the onset of disease.

Hematologic Effects

Hematologic effects of 60 and 120 mg/kg of cyclophosphamide have been previously reported in detail (16,17). After 12 courses of 60 mg/kg of cyclophosphamide the white blood cell (wbc) count fell below 1000 cells/mm^3 by Day 6 (range 4–9) and rose to greater than 1000 cells/mm^3 by Day 15 (range 12–20) with a median nadir of 200 cells/mm^3 (range 40–270). The median platelet nadir was 90,000 and fell below 50,000 platelets/mm^3 in three instances. After 120 mg/kg the wbc count fell below 1000 cells/mm^3 by Day 7 (range 4–8) and recovered to over 1000 cells/mm^3 by Day 17 (range 11–24) with a median nadir of 30 cells/mm^3 (range 0–100). All patients had platelet counts below 50,000 platelets/mm^3. The platelet level fell below 100,000 platelets/mm^3 by Day 8 (range 5–12) and recovered to above this level by Day 17 (range 10–27) with a median nadir of 19,000 platelets/mm^3 (range 6400–37,000). There was a tendency for delayed platelet recovery with subsequent courses as compared with the first course, but leukocyte recovery was the same on repeated courses.

Infection and Fever

Fever greater than 101 F occurred in the patients after three of 12 courses of 60 mg/kg. One patient who had previously received 120 mg/kg had a localized staphylococcal infection for which antibiotics were given. Fever occurred after 17 of 18 courses of cyclophosphamide (120 mg/kg) and lasted a median of 5 days (range 2–13). Localized infection, usually of skin, pharynx, or perianal area, occurred after 15 of 18 courses of the drug at 120 mg/kg and was associated with septicemia in four instances. Antibiotics were administered after these 15 courses and one additional course without documented infection. Infections and fever cleared with the return of granulocyte counts.

Transfusions

Platelet transfusions were not required for patients receiving 60 mg/kg of cyclophosphamide. Patient No. 8 required red blood cell (rbc) transfusions after both courses of 60 mg/kg. Platelets were administered after nine of 18 courses of cyclophosphamide (120

mg/kg) when platelet counts fell below 20,000 platelets/mm³. There were no episodes of significant hemorrhage. Rbc transfusions were administered to one patient.

Nonhematologic Toxic Effects

Mild symptomatic cystitis was seen after administration of two of 12 courses at 60 mg/kg of cyclophosphamide and after three of 18 courses at 120 mg/kg and was associated with microscopic hematuria. All symptoms cleared prior to subsequent courses with no indication of repeated episodes. Total or near total scalp alopecia developed in all patients at both dose levels. No hepatic or renal toxic effects were detected at either dose level. Patient No. 9 had first-degree heart block, cardiomegaly, and congestive heart failure associated with a *Streptococcus viridans* septicemia after his third course of cyclophosphamide. These cardiac complications improved with clearing of infection and return of marrow function. His cardiac function was normal 24 months after therapy. There was no evidence of cardiac toxic reactions in any of the other patients at either dose level.

DISCUSSION

Cyclophosphamide given in intermittent doses of 15–40 mg/kg is well tolerated without severe or prolonged pancytopenia (15,19, 20). Increasing the dose to 60 mg/kg produced more severe pancytopenia, but without a significant incidence of infection or a dangerous reduction in platelet levels (16). Increasing the dose still further to 120 mg/kg increased the severity but not the duration of pancytopenia (16). At this dosage level 50% of the patients had local infections with a 20% incidence of septicemia and 50% required platelet transfusion to maintain platelet levels above 20,000 platelets/mm³. An intermittent administration of 120 mg/kg of cyclophosphamide to patients with ovarian carcinoma demonstrated that the primary cumulative toxic manifestation was delayed platelet recovery (17).

In the present study, median nadir wbc and platelet counts were somewhat lower at both dose levels as compared to a previous study (16). The infectious complications at 60 mg/kg were insignificant in both studies. Eighty-four percent of patients receiving 120 mg/kg in this study had infections as compared to 50% previously reported (16), with the frequency of septicemia remaining the same. This apparent increase may reflect the small number of patients involved, or it may reflect the fact that four patients had received prior radiation therapy and one had received both radiation therapy and chemotherapy.

Nonmarrow toxic effects were limited to nausea and vomiting, total scalp alopecia, and an occasional episode of cystitis. Liver and renal toxic effects were not seen. One patient (No. 9) had cardiomegaly and congestive heart failure during the pancytopenic phase after his third course of cyclophosphamide. This occurred at a time when he was profoundly granulocytopenic and infected, and the contribution, if any, of this drug to his cardiac problem remains speculative. In man, cardiac toxicity has been reported after acute doses of 240 mg/kg (16) and has been suspected after doses of 200 mg/kg (21,22). In addition, such toxicity has been well documented in nonhuman primates after doses of 240 mg/kg (23). Cumulative cardiac toxicity was not seen when doses of 60–120 mg/kg were given at 4–6-week intervals for four to six courses (17).

Although the numbers are small, the response rate in this study (seven of nine patients) is higher than in most series. As with other regimens, the responses were of short duration in most instances and the median survival time was less than 12 months. Two of nine patients achieved a complete remission and one patient has been alive and free of disease for over 2 years. The other patient unfortunately refused further therapy after achieving a complete response and relapsed in 4 months.

It was apparent from this study that intermittent high-dose cyclophosphamide was inadequate to control disease in the majority of patients, although all patients showed some and most patients showed dramatic initial tumor reduction. Unfortunately, relapses occurred between courses even when the inter-

val between treatments was as short as 3 weeks. Currently, we are evaluating the addition of nonmarrow toxic agents (vincristine, mithramycin, bleomycin) to high-dose cyclophosphamide (120 mg/kg) and actinomycin D in an attempt to prevent recurrences. Three of five patients treated on this new protocol have achieved complete responses but further studies will be necessary for evaluation.

LIST OF COMPOUNDS

Actinomycin D: NSC-3053

Bleomycin: NSC-125066

Cyclophosphamide: CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8

Mithramycin: NSC-24559; CAS reg. No. 18378-89-7

Sarcolysin: NSC-14210; phenylalanine mustard; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, DL-

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate

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Guanazole (NSC-1895) in the Treatment of Childhood Leukemia [1,2,3]

Vita J. Land, John M. Falletta, Campbell W. McMillan, and Thomas E. Williams [4,5,6]

SUMMARY

Twenty-six children with late-stage acute leukemia in relapse were treated with a total of 48 courses of guanazole at a dose of 15 g/m²/day for 5 days, given approximately every 2 weeks. No complete or partial remissions were achieved in 18 evaluable patients, although two of three evaluable patients with acute myelogenous leukemia and one of 15 evaluable patients with acute lymphoblastic leukemia showed a transient decrease in the number and percentage of blast cells in the bone marrow and peripheral blood. Bone marrow depression, attributed to the guanazole therapy, occurred in all but six evaluable patients.

[Cancer Chemother Rep 58:715–717, 1974]

Guanazole resembles hydroxyurea [7] in its mechanism of action (probably acting by inhibiting ribonucleotide reductase and thus DNA synthesis) and has activity against L1210 leukemia (1). Preclinical toxicity studies of this agent have been done in rodents, dogs, and rhesus monkeys [8] together with in vivo studies of drug distribution in rodents, dogs (2), and subsequently man (3). Most recently, the earliest clinical trials using guanazole in the treatment of acute leukemia (4,5) and solid tumors (4) [9] have been carried out. The objective of the present study conducted by the Pediatric Division of the Southwest Oncology Group was to determine whether guanazole had activity in leukemia in children.

METHODS

Twenty-six patients (13 girls and 13 boys) under 17 years of age when diagnosed as having acute leukemia were included in the

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[2] Supported by Public Health Service grants CA-05587, CA-11232, CA-03161, CA-04919, CA-12644, and CA-12014 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3] Guanazole: CAS reg. No. 503-88-8; 1,2,4-triazolidine, 3,5-diimino-. Supplied by Cancer Therapy Evaluation, Division of Cancer Treatment (DCT), NCI.

[4] This study was done by the Southwest Oncology Group. The following institutions participated in the study: Washington University School of Medicine, St. Louis, Mo (*Vita J. Land, Abdel H. Ragab,* and *Teresa J. Vietti*); Emory University School of Medicine, Atlanta, Ga (*Owen Grush*); University of Texas Medical School at San Antonio (*Thomas E. Williams*); University of Mississippi School of Medicine, Jackson (*Jeanette Pullen*); University of Oklahoma School of Medicine, Oklahoma City (*G. Bennett Humphrey* and *Ruprecht Nitschke*); Baylor College of Medicine, Houston, Tex (*Donald J. Fernbach* and *Kenneth A. Starling*); Medical University of South Carolina, Charleston (*Samuel K. Morgan*); University of North Carolina School of Medicine, Chapel Hill (*Campbell W. McMillan*); and Cleveland Clinic, Ohio (*Paul G. Dyment* and *Derrick Lonsdale*).

[5] The authors wish to thank Drs. Barth Hoogstraten and Teresa Vietti for their critical review of the manuscript.

[6] *Reprint requests to*: Vita J. Land, MD, Department of Pediatrics, Washington University School of Medicine, 500 South Kingshighway, St. Louis, Mo 63110.

[7] Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1.

[8] Livingston RB, and Carter SK. Clinical brochure: guanazole (NSC-1895), Nov 1969. Prepared by the DCT, NCI, Bethesda, Md.

[9] Vaitkevicius VK. Southwest Oncology Group. Unpublished data.

study. All were resistant to standard chemotherapeutic agents and had no evidence of azotemia (serum creatinine greater than 1.0 mg/100 ml with a surface area greater than 1.5 m^2, or greater than 0.6 mg/100 ml with a surface area less than 1.5 m^2). After informed consent was obtained, each patient was given guanazole in a dose of 15 g/m^2/day for 5 days as a continuous intravenous (iv) infusion (table 1). A second course was started 13–22 days after Day 1 of the first course in 18 patients, and a third course was started 23–33 days after Day 1 in four patients. One patient received a fourth course of guanazole 35 days after Day 1. Dosage was modified for the second and subsequent course depending upon the bone marrow cellularity. Only those patients who received two or more courses of guanazole therapy were considered evaluable for response.

RESULTS AND DISCUSSION

(See table 1)

One of 15 evaluable patients with acute lymphoblastic leukemia (ALL) and two of three evaluable patients with acute myelogenous leukemia (AML) showed a decrease in the percentage of blast cells in the bone marrow or peripheral blood. There were no partial or complete remissions or disappearances of peripheral blast cells. Six patients, including two with AML, were inevaluable because of early death that occurred 1–8 days after guanazole therapy was started, and one patient was inevaluable because of an inadequate trial due to suspected renal toxicity which was not confirmed by biochemical or postmortem examination. Bone marrow depression was thought to be present in 12 patients, but in five patients we

TABLE 1.—Effects of guanazole therapy

Patient No.	No. of prior remissions	Diagnosis*	Days from diagnosis to start of guanazole treatment	Days from end of guanazole treatment to death	No. of guanazole courses	Objective response†	Toxic effects‡
1	3	ALL	742	9	<1	↓ Blasts	? Renal
2	5	ALL	2233	11	2	Disease progression	None
3	3	ALL	263	149	3	None	Pancytopenia
4	2	ALL	311	9	2	None	Pancytopenia
5	6	ALL	1277	3	2	None	? Liver
6	4	ALL	1201	197	2	None	Pancytopenia
7	3	ALL	1081	51	3	None	None
8	2	ALL	549	8	1	↓ Blasts	↓ Wbc
9	5	ALL	415	39	3	None	Pancytopenia
10	3	ALL	591	1	1	↓ Blasts	None
11	2	ALL	428	44	2	None	↓ Wbc
12	5	ALL	1296	10	2	None	Pancytopenia
13	7	ALL	2477	18	2	None	None
14	7	ALL	1105	3	2	None	Pancytopenia
15	3	ALL	505	1	1	None	None
16	3	ALL	1519	16	2	None	None
17	1	ALL	154	49	3	↓ Blasts	Pancytopenia
18	2	ALL	253	1	2	Disease progression	↓ Wbc
19	2	ALL	625	1	<1	None	None
20	1	ALL	175	79	1	Disease progression	↓ Wbc, vomiting
21	2	AML	469	8	2	↓ Blasts	None
22	2	AML	768	7	4	↓ Blasts	Pancytopenia
23	3	AML	521	6	2	Disease progression	None
24	1	AML	445	1	<1	None	None
25	1	AML	182	2	1	None	None
26	2	AML	204	6	2	None	None

*ALL = acute lymphoblastic leukemia; AML = acute myelogenous leukemia.
† ↓Blasts = decrease in blast cells.
‡ ↓Wbc = decrease in white blood cells.

could not define whether this effect was due to guanazole therapy or to bone marrow infiltration by leukemia. The dosage was reduced to 10 g/m²/day for the second course and to 6 g/m²/day for the third course in one patient because of marked marrow hypoplasia that was associated with peripheral pancytopenia and a decrease in blast cells. The dosage was increased to 20 g/m²/day in two patients for the second course, in three patients for the third course, and in one patient for the fourth course because of a hyperplastic marrow with no antileukemic response. The median time to death was 8 days (range 1–197 days) after guanazole therapy was discontinued.

Yakar and his colleagues (4) administered guanazole by continuous iv infusion in 5-day courses to 27 patients with malignancies. Myelosuppression was the major toxic effect. Complete remissions were achieved in two of the 14 patients with previously treated AML, lasting 16 months and 11 weeks, respectively. A very brief complete remission was obtained in one of four patients with advanced ALL. All remissions occurred at doses of 25–40 g/m²/day given for 5 days.

Hewlett and his associates of the Southwest Oncology Group (5) treated 44 evaluable patients (all adults) with acute leukemia—30 with AML, seven with chronic myeloblastic leukemia (CML) (blast cell transformation), and seven with ALL. A complete remission lasting 49–451 days occurred in three patients with AML. One patient with CML achieved a complete remission lasting 99 days. There were no complete remissions in patients with ALL. Although the major toxic effect was myelosuppression, no major difference was apparent between the degree of myelosuppression at doses between 15 and 30 g and at individual doses ranging from 7.5 to 10 g. Fever was seen less frequently in the patients in Hewlett's study than in the patients reported by Yakar et al (4).

The Southwest Oncology Group [10] evaluated guanazole in phase I studies of 82 patients with various solid tumors who were given a total of 85 courses. Hematologic toxic effects were seen in most patients who received 5 g/m²/dose or more. Mild nausea, and occasional fever, stomatitis, and somnolence were also noted. Two responses were noted in a total of 69 evaluable patients. These responses were seen in one patient with melanoma (who received 10 g/m²/day × 5) and in one patient with carcinoma of the prostate (who received 6 g/m²/day × 5). The same investigators also evaluated guanazole given in a bolus iv twice weekly for 3 weeks in a phase I study, with a response observed in one patient with carcinoma of the lungs (treated with 10 g/m² twice weekly for 6 weeks). Myelotoxic effects and fever were less frequent at this dose schedule.

The absence of a complete or partial remission among 18 evaluable patients in the present study indicates a response rate of less than 20% at 95% confidence limits in patients treated at 15 g/m²/day for the first course (6). Thus, it appears that guanazole used against ALL in children merits possible pursuit only in combination with other chemotherapeutic agents (further dose escalation in ALL being limited by toxic effects in our study) or as a single agent used against AML at dose levels in excess of those used in the present study.

[10] *See* footnote 9.

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Response of Childhood Histiocytosis X to Procarbazine (NSC-77213) [1,2]

Diane M. Komp, Howard A. Britton, Teresa J. Vietti, and G. Bennett Humphrey [3,4]

SUMMARY

Nine previously treated children with histiocytosis X were treated in a phase II study with procarbazine at an initial dose of 50 mg/m²/day orally. Observed toxic effects included bone marrow depression and vomiting. Bone marrow toxic manifestations necessitated discontinuation of therapy in two of ten patients. A complete response was seen in one of ten patients, a partial response in four of ten, a mixed response in four of ten, and no response in one of ten. It was concluded that procarbazine is useful in the treatment of histiocytosis X. Clinical trials of procarbazine in combination with other agents are in progress.

[Cancer Chemother Rep 58:719–722, 1974]

Histiocytosis X is an uncommon disorder marked by variable clinical signs and effects (1). The most virulent forms of this disease are usually seen in children under 2 years of age at the time of diagnosis and children with multiple organ involvement (2). Chemotherapeutic responses have been reported most often with the following classes of agents: corticosteroids (3), alkylating agents (1), vinca alkaloids (1), and antifolates (3).

Procarbazine, a methylhydrazine derivative, has shown considerable activity in Hodgkin's disease with response rates in various series ranging from 60% to 75% (4–7), but remissions have been of short duration (8). Lack of cross resistance with vinca alkaloids and alkylating agents has been prominent in all series. This study was initiated to determine the response rate in children with histiocytosis X who were resistant to standard chemotherapeutic agents.

MATERIALS AND METHODS

Patient Selection

Children younger than 15 years of age with biopsy-proven histiocytosis X were eligible for a phase II study of procarbazine if they had had prior treatment with vinca alkaloids, alkylating agents, and corticosteroids and either failed to achieve complete responses with any of these agents or relapsed during or after such treatment.

No distinction was made between Hand-Schüller-Christian and Letterer-Siwe syn-

[1] Received Mar 28, 1974; accepted May 23, 1974.

[2] Procarbazine: CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride; Matulane. Supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, National Cancer Institute (NCI).

[3] This study was done by the Southwest Oncology Group; CA-12644. The following institutions participated in the study: University of Texas Medical School, San Antonio (*Thomas E. Williams* and *Howard A. Britton*); Washington University School of Medicine, St. Louis, Mo (*Teresa J. Vietti, Vita J. Land,* and *A. H. Ragab;* CA–05587); University of Virginia School of Medicine, Charlottesville (*Diane M. Komp;* CA–08223); Wilford Hall Hospital, Lackland Air Force Base, San Antonio, Tex (*Frederick Koch*); University of Oklahoma School of Medicine, Oklahoma City (*G. Bennett Humphrey* and *Ruphrect Nitschke*; CA–11233). Supported by Public Health Service grants from the NCI, National Institutes of Health, Department of Health, Education, and Welfare.

[4] *Reprint requests to:* Southwest Oncology Group, Operations Office, 3500 Rainbow Blvd, Suite 201, Kansas City, Kan 66103.

dromes. All children had active disease manifested by progression of lesions. Patients receiving radiotherapy to the only measurable lesions were excluded.

Informed consent was obtained according to institutional guidelines.

Study Regimen

Procarbazine was initially given in a single daily dose of 50 mg/m^2 orally. If this dose was tolerated by the patients without undue toxicity, it was escalated in increments of 25 mg/m^2. Phenothiazine derivatives were avoided for the treatment of emesis.

Patients were removed from the study if by 8 weeks there was no response or if the disease had progressed. At the discretion of the investigator patients with rapid disease progression were removed from the study earlier. Patients who responded remained on therapy until disease recurrence or intolerable toxic reactions necessitated discontinuation.

Modification

Dosage was reduced by one half if there was an absolute granulocyte count of <1000 cells/mm^3, a platelet count of <75,000 platelets/mm^3, or severe nausea unrelieved by antiemetics. Procarbazine was discontinued if there was an absolute granulocyte count of <500 cells/mm^3, a platelet count of <50,000 platelets/mm^3, stomatitis, diarrhea, severe paresthesias, convulsions, neuropathies, confusion, or severe nausea unrelieved by antiemetics. After the toxic effects had disappeared, treatment was resumed at one half the dose that caused the toxicity. Patients experiencing hypersensitivity were permanently removed from the study.

Criteria for Response

All measurable lesions were recorded at the time of registration. Complete response (CR) was defined as the healing of measurable lesions with no new lesions appearing. Lack of improvement in diabetes insipidus did not prevent qualification for CR. Partial response (PR) was defined as a 50% reduction in all measurable lesions. Mixed response (MR) was defined as an improvement in one area of involvement with progression of disease elsewhere. Children who failed to improve or had progressive disease were classified as nonresponders (NR).

RESULTS

Response

Eleven children were registered for the study. One child was considered ineligible for evaluation because of inadequate histologic evidence of histiocytosis X. One child (patient No. 3), for whom procarbazine was discontinued because of toxicity, received a second trial for a total of ten trials in nine evaluable patients. At the time of treatment, the patients' ages ranged from 16 months to 10$\frac{5}{12}$ years. The characteristics and prior therapy of the patients are summarized in table 1.

Eight of nine patients achieved at least an MR to treatment. The average time to maximum response was 22 days with a range of 10–47 days. The duration of maintained remission ranged from 12 to 97 days with a mean of 49 days. Two patients, for whom toxicity necessitated discontinuation of therapy, subsequently had increasing disease. Patient No. 3 had a second PR when a second trial was given.

Only one patient (No. 1) achieved CR status. The remainder of the patients' responses were four of ten PR, four of ten MR, and one of ten NR. The most favorable response was seen in the oldest child. The nonresponder was 28 months old at the time of treatment.

Dosage and Toxic Effects

Table 1 summarizes the dosage, response, and toxic effects for the ten evaluable trials. Escalation of dose was possible in only two patients. Treatment was interrupted for intercurrent infection in two patients and for vomiting in two other patients. Hypersensitivity, paresthesia, neuropathy, confusion, or hemolytic anemia were not seen. One child, who also had liver failure, had convulsions. Bone marrow suppression was seen in four of ten trials; vomiting in two of ten trials; diarrhea in one of ten trials; and stomatitis in one of ten trials. Bone marrow suppression was severe enough

TABLE 1.—History, clinical findings, responses, and toxic effects in patients treated with procarbazine

Patient No.	Age at diagnosis (mos)	Duration of disease (yrs)	Site of organ involvement	Therapy (mg/m²/ No. of days)	Response			Toxic effects
					Type*	Time to maximum (days)	Length maintained/ unmaintained (days)	
1	101	2	Spleen Skin Parotid Liver	50/119	CR	14	95/0	Moderate vomiting Moderate leukopenia
2	27	1⅛	Skull Scalp	50/54	PR	47	28/0	Moderate vomiting
3 (trial 1)	8	8⅔	Skull Liver Spleen Nodes	50/71	PR	29	42/56	Moderate diarrhea
3 (trial 2)		9½	Liver Spleen Nodes	50/59	PR	35	14/7+	Severe stomatitis Severe leukopenia
4	31	7/12	Liver Spleen Nodes	50/36	PR	22	21/0	None
5	8	2⅓	Scalp Liver Spleen	50/108	MR	11	90/0	None
6	15	1⅓	Skull Liver Spleen Nodes	50/14 75/35 100/62	MR	14	97/24	Severe pancytopenia
7	3	4½	Skull DI † Pelvis Skin	50/7 DC/14 50/9 75/14 100/14	MR	10	37/0	None
8	5	11/12	Skull Skin Liver Nodes	50/40	MR	20	12/0	Severe leukopenia Convulsions?
9	23	5/12	Skin Ears	50/35	NR	—	—	None

* CR = complete response; PR = partial response; MR = mixed response; NR = no response.
† DI = diabetes insipidus; DC = discontinued.

TABLE 2.—Comparison of responses to procarbazine with responses to vinca alkaloids and alkylating agents in the Southwest Oncology Group

Drug	No. of patients treated	No. of trials	No. of responses				Total PR + CR
			NR	MR	PR	CR	
Vincristine	6	6	3	0	0	3	3 (50%)
Vinblastine	20	20	6	3	4	7	11 (55%)
Cyclophosphamide	22	22	7	1	5	9	14 (63%)
Procarbazine	9	10	1	4	4	1	5 (50%)

to discontinue therapy in two patients. Four patients experienced no toxic effects.

DISCUSSION

Because of the extensive prior treatment of patients in this study, there is no reported group of children with histiocytosis X in the literature with which this study group can be compared. The range of CR and PR for previously untreated or minimally treated children in the Southwest Oncology Group using vincristine, vinblastine,[5] and cyclophosphamide[6] is 50%–63% (1) compared to 50% for procarbazine (table 2).

Procarbazine appears to have activity in the treatment of histiocytosis X. The quality of response as evaluated by the type and length of response was shallow. Toxicity is significant.

Because of extensive prior treatment, these patients' responses may not be representative of responses in previously untreated patients. Similar quality of response is seen with procarbazine in the treatment of Hodgkin's disease. Trials of a MOPP-type[7] regimen in advanced histiocytosis X are now in progress.

[5] Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate.

[6] Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate.

[7] MOPP = mechlorethamine, vincristine (Oncovin), prednisone, and procarbazine.

Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride.

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Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1); Oncovin.

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-.

Clinical Pharmacology of Oral and Intravenous 5-Fluorouracil (NSC-19893) [1,2,3,4]

J. L. Cohen, L. E. Irwin, G. J. Marshall, H. Darvey, and J. R. Bateman [5,6,7]

SUMMARY

The comparative clinical pharmacology of oral and intravenous (iv) 5-fluorouracil (5-FU) was studied by observing the disposition of the free drug in 19 patients with disseminated adenocarcinoma after a single 15-mg/kg/week dose. Seven patients were studied after receiving 5-FU iv and all showed consistent and rapid plasma clearance of the drug with an average plasma half-life of 10 minutes. Ten patients studied after oral ingestion of 5-FU in water demonstrated widely varying peak plasma concentrations (2–40 μg/ml) with measurable plasma levels being maintained from 4 to 12 hours. 5-FU (from an oral dose) was found to have a 50% and 80% availability in two different patients receiving oral and iv doses on consecutive weeks. In two of three patients receiving this drug orally on consecutive weeks (in different vehicles) there was a significant increase in availability when given in a basic bicarbonate vehicle and water compared with administration in orange juice. Serial bone marrow aspirations were obtained concurrently with plasma samples after oral and iv doses in a selected number of patients and the uptake of radiolabeled thymidine and deoxyuridine by these marrow samples was compared to the corresponding plasma levels of the drug.

[Cancer Chemother Rep 58:723–731, 1974]

5-Fluorouracil (5-FU) is a well-established antimetabolite used in the treatment of several types of disseminated malignancies (1,2). Although the mechanism of action of 5-FU has been known for several years (3), the clinical response has not been consistent and the results have not led to a universally accepted treatment schedule for any type of malignancy. Rather, different investigators and institutions administer 5-FU in accordance with their own experience using various doses and schedules. It is usually administered intravenously (iv) and numerous reports have documented the activity and toxicity of the different treatment schedules in various types of malignancies (4–8).

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[2] Supported by Public Health Service grants CA-05186 from the National Cancer Institute and RR5702, both from the National Institutes of Health, Department of Health, Education, and Welfare.

[3] This study was done by the Western Cancer Study Group (WCSG).

[4] 5-Fluorouracil: CAS reg. No. 51-21-8; 5-FU. Supplied by Hoffmann LaRoche, Nutley, NJ.

[5] Departments of Medicine and Pharmacy, University of Southern California, Los Angeles.

[6] *Reprint requests to:* Joseph R. Bateman, MD, University of Southern California, School of Medicine, 2825 S Hope St, Los Angeles, Calif 90007.

[7] The support of Dr. Richard Staab and the technical assistance of Patrick Brennan, Brian Gourley, and Linda Keller are gratefully acknowledged.

5-FU has also been administered in capsules, tablets, suspension, and solution, and has demonstrated more significant activity and somewhat modified toxicity than the iv route (9–11). A recent review by Ansfield (12) discusses the current status of 5-FU therapy and emphasizes the need for further systematic study of this agent without compromising the treatment of patients.

It would be of value to systematically observe the disposition of 5-FU as a function of these various dose schedules and routes of administration in several patients with responsive diseases and attempt to correlate this disposition with observable clinical responses. This is especially complex in the case of 5-FU because of its rapid and extensive anabolic and catabolic alteration which accounts for its activity, toxicity, and elimination from the body. In addition, wide variation in patient response makes the study of more than a few patients essential to determine an "average" set of pharmacokinetic parameters which could be useful in constructing a model for the drug and optimizing therapy.

The absence of detailed pharmacokinetic and clinical pharmacologic studies on 5-FU in the literature is attributable to the complex metabolism of the drug and the absence of a rapid, sensitive, analytical method for the determination of free 5-FU in large numbers of biologic samples. Mukherjee et al (13,14) looked at the disposition of 5-FU-2-C^{14} after iv, intramuscular, intraperitoneal, and oral administration in a limited number of patients, but measured only total radioactivity in the plasma and distinguished between free 5-FU and its catabolic products, urea and α-fluoro-β-ureidopropionic acid, in urine. Clarkson et al (15) utilized a sensitive microbiologic procedure to study the disposition of oral and iv 5-FU in three patients. The development of a suitable gas chromatographic method of analysis for free 5-FU in our laboratories (16) has enabled us to study the disposition of this drug in several patients receiving 5-FU therapy.

The present study describes the disposition of 5-FU in 19 patients with disseminated adenocarcinoma. Because of the significant interest in oral therapy and the availability of patients through Western Cancer Study Group (WCSG) protocol 106, we were particularly concerned with comparing iv and oral administration of 15-mg/kg/week doses. We also studied the pharmacologic availability of 5-FU after an oral dose, the effect of the vehicle on the availability of an oral dose, the relative physiologic disposition of an oral dose in the presence and absence of liver malignancy, and the concurrent measurement of the inhibition of uptake of C^{14}-thymidine and C^{14}-deoxyuridine as a measure of bone marrow cell toxicity.

MATERIALS AND METHODS

All but five of the patients studied qualified for the randomized iv-oral 5-FU WCSG protocol 106, the details of which have been reported previously (10). The requirements for all patients studied were the same and briefly included: disseminated measurable adenocarcinoma with histologic confirmation, no prior 5-FU therapy, and a treatment interval of 3 weeks after irradiation or other prior chemotherapy. Five patients received drug by a dose schedule or a route of administration which did not follow the protocol design, ie, three patients received oral doses on consecutive weeks in different vehicles (rather than in distilled water as required) and two patients received an oral dose on the first week, followed by an iv dose the next week. All patients reported in this paper were studied after receiving the initial dose in their therapy program (unless otherwise indicated).

Patients received a single 15-mg/kg dose based upon ideal body weight, actual weight, or estimated dry weight (whichever was less) either orally or by iv push. Commercially available 5-FU in 10-ml ampules containing 500 mg of 5-FU was dissolved in 4 ounces of distilled water for oral administration. For studies concerned with the effects of the vehicle on oral absorption, orange juice or 0.2 M pH 9 bicarbonate buffer was substituted for distilled water. Patients receiving iv therapy were given their usual diet, but patients receiving oral therapy were not allowed to eat from 2 hours before to 2 hours after ingesting the drug.

After iv administration of 5-FU, venous blood samples (at least 4 ml) were taken at 0, 2, 4, 8, 15, 30, 60, 120, and 180 minutes and at 12 and 24 hours. After oral administration of 5-FU these samples were taken at 0, 15, 30, 45, and 60 minutes and sometimes after 48 hours. The samples taken from 0 to 3 hours were collected through an indwelling catheter and those taken after longer intervals were collected by venipuncture. Blood samples were stored in Vacutainers[8] containing either citrate or EDTA to prevent clotting. These were immediately refrigerated and then centrifuged as soon as possible to allow separation of the plasma, which was either directly analyzed or frozen. Urine specimens were collected in separate bottles under toluene as voided for 12 hours from the patients given the iv dose and for 24 hours from the patients given the oral dose.

The analytical procedure involves the saturation of a 1.0-ml plasma sample with solid sodium sulfate and subsequent extraction with a 16% *n*-propanol-ether mixture. The organic layer is evaporated until dry and the residue silylated using 100 μl of a 50% solution of *N,O*-(bis)-(trimethylsilyl)-trifluoroacetamide (BSTFA, Pierce Chemical Co., Rockford, Ill) in pyridine. The ditrimethylsilyl analog formed is chromatographed on a Varian model 1440 gas chromatograph with an OV-1 glass column using flame ionization detection. An internal standard, either 5-chlorouracil or anthracene, is used to quantitate the results. The method is rapid, specific for free 5-FU, and has a maximum sensitivity of 0.5 μg/ml in plasma. Urine samples were lyophilized to reduce the volume to about 5 ml. These were then made alkaline by the addition of 0.01 N NaOH and were preextracted with ether. The aqueous phase was then removed, the pH was adjusted to 6, and the above analytical procedure was performed. The specific details of the analysis are published elsewhere (16).

The effects of 5-FU on bone marrow were studied concurrently with the plasma concentration in two patients who received the drug iv and four patients who received it orally. Iliac marrow samples (1–2 ml) were obtained before drug administration and at 30 minutes and 3, 6, 24, and 48 hours after treatment. The marrow specimens were suspended in 7 ml of cold Robinson's medium. Using a Pasteur pipet the mixture was vigorously agitated in order to obtain as uniform a suspension of cells as possible. Aliquots (3.0 ml) were pipetted into each of two 25-ml flasks, one which contained 0.1 microcurie of C^{14}-thymidine (specific activity 55 microcurie/μmol) and the other which contained 0.1 microcurie of C^{14}-deoxyuridine of the same specific activity. The flasks were incubated in a 37 C water bath and shook for 40 minutes. The DNA was extracted by standard methods and quantitated from aliquots measured at 260 nm on a Hitachi spectrophotometer. Four aliquots of 0.1 ml of the extracted DNA were spotted on planchet and the activity was determined on a gas-flow counter. Four hundred counts were obtained on all samples and the counts/minute/μg of DNA were calculated and plotted.

RESULTS

Iv Studies

As shown in figure 1, 5-FU disappears very rapidly from the plasma after a single 15-mg/kg iv dose. After an extremely short distribution phase, the drug appears to follow a single compartment kinetic model with a mean plasma half-life of 10 minutes. Table 1 summarizes the data for the seven patients who were studied after receiving an iv dose of 5-FU. In all cases (regardless of disease type) there was a rapid clearance of drug with no measurable plasma levels occurring after 90 minutes. The volume of distribution of 0.12 liter/kg was calculated from the extrapolated intercept of the linear portion of log concentration × time (C × T) plot and ignores the initial distribution phase. The estimated clearance of 8.4 ml/min/kg probably represents mainly metabolism since within the sensitivity limits of the assay, no free 5-FU was found in the urine, which was collected in as many fractions as possible during the study.

Oral Studies

The ten patients who received a single 15-mg/kg oral dose of 5-FU in solution showed

[8] Becton-Dickinson Co., Rutherford, NJ.

widely variable plasma C × T curves. Figure 2 describes the extremes after oral ingestion. Peak plasma levels, which occurred from 30 minutes to 2 hours after drug administration, ranged from 2 to 40 μg/ml. In all cases the higher the peak plasma level the earlier the peak occurred and the more rapidly it disappeared. Residual measurable amounts of free 5-FU occurred in the plasma from 4 to 12 hours after drug administration. This suggests slow or prolonged absorption and perhaps biliary recycling. The wide interpatient differences also indicate significant variation in the absorption of 5-FU from an oral dose. In two selected patients there was no measurable free drug in the fractionally collected urine obtained during the study. Table 2 summarizes the data of the ten patients who received the drug orally with the patient population being divided according to the presence or lack of hepatic involvement (either primary or metastatic) without regard to primary tumor type. These data indicate (at least in a limited number of patients) that patients with hepatic tumor or

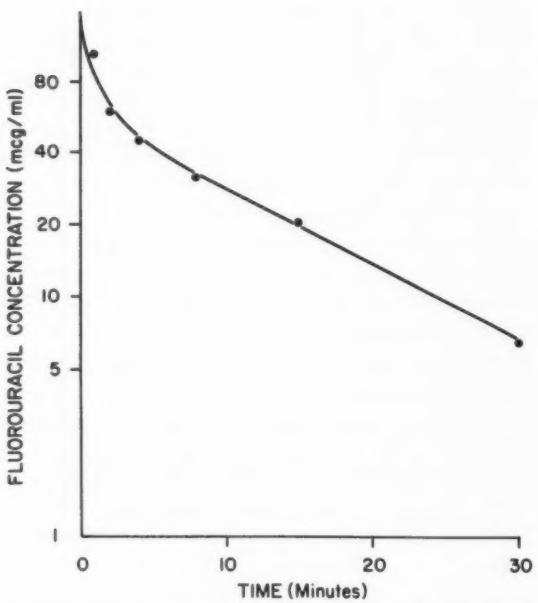

FIGURE 1.—Plasma concentration of 5-FU in a patient after a single iv dose of 15 mg/kg was given (patient No. 9—bile duct carcinoma metastatic to the liver).

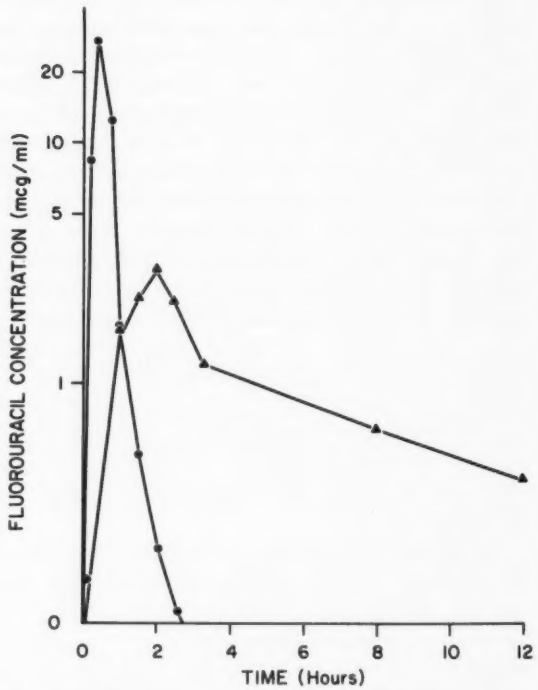

FIGURE 2.—Plasma concentration of 5-FU in two different patients after a single 15-mg/kg oral dose was given in water. ● = Patient No. 1—carcinoma of the colon; ▲ = patient No. 10—metastatic carcinoma.

metastases do not achieve as high a blood level of 5-FU but show continuous levels for much longer times than do patients with no hepatic involvement who appear to "handle" both oral and iv doses in the same way.

Vehicle Studies

Because of the poor palatability of an orally administered aqueous solution of 5-FU, out-

TABLE 1.—Summary of iv pharmacokinetic data*

No. of patients	7
Plasma half-life†	10.0 ± 2.1 min
Initial extrapolated plasma concentration	60–100 μg/ml
Volume of distribution	0.12 liter/kg
Total estimated clearance	8.4 ml/min/kg

*All patients received 15 mg/kg 5-FU by iv push.
†Mean ± standard deviation.

TABLE 2.—Summary of oral disposition data*

	No. of patients	Peak plasma concentration† (μg/ml)	Time of peak plasma concentration (min)	Latest appearance of measurable plasma levels (hrs)
Hepatic involvement	6	3.0 ± 1.2	100 (70–120)	14 (6–24)
No hepatic involvement	4	22.2 ± 5.3	35 (30–45)	6 (4–7)

*All patients received 15 mg/kg 5-FU orally in distilled water.
†Mean ± standard deviation.

patients have reported using various vehicles to mask the taste. Most often mentioned were orange or other citrus juices. Because of the pKa of the drug, the relatively high dose, and the limited water solubility, the effect of the pH of the vehicle on the drug absorption was studied in three patients. Consecutive weekly oral 15-mg/kg doses of 5-FU diluted in orange juice, water, and pH 9 bicarbonate buffer were administered and the plasma C × T curves were compared (fig 3). Two of these patients had greater apparent drug availability from the alkaline medium and from water than from the orange juice. The third patient evidenced the highest availability with water and the lowest with bicarbonate. To obtain a measure of individual variation in absorption, independent of vehicle, a fourth patient received consecutive weekly doses in water and demonstrated no significant differences in the plasma levels.

Availability Studies

Two patients were studied after they received the drug by both routes on consecutive weeks to compare the total area under the plasma C × T curve after oral and iv doses. Figure 4 illustrates the disposition of both doses in each of these patients. Since one of these patients (No. 2) had metastases to the liver and the other patient (No. 1) did not, the total area

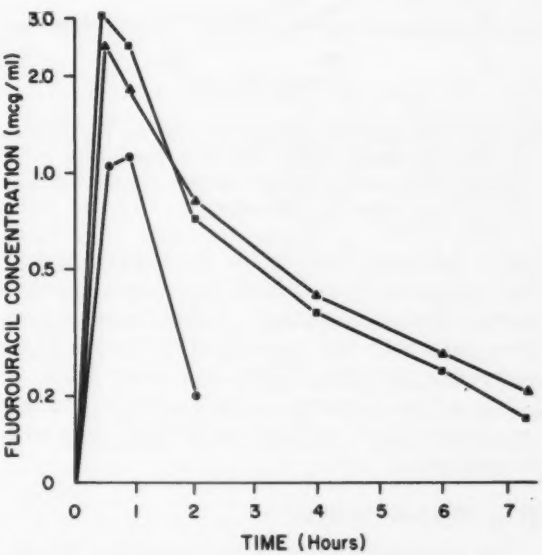

FIGURE 3.—Plasma concentration curves for 5-FU after single oral doses of 15 mg/kg given in different vehicles. ■ = pH 9 $NaHCO_3$ solution; ● = orange juice; ▲ = distilled water (patient No. 4—carcinoma of the rectum metastatic to the liver).

TABLE 3.—Summary of availability data

Patient No.	Disease	Order of doses	Availability (relative C × T)
1	Carcinoma of the colon	Oral-iv	80%
2	Carcinoma of the colon, metastatic to liver	Iv-oral	50%

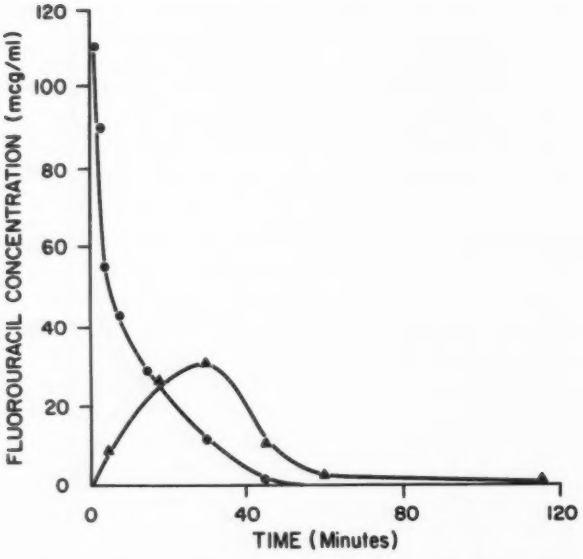

FIGURE 4.—Plasma concentration curves for 5-FU after oral and iv doses given to the same patient. ● = 15 Mg/kg iv; ▲ = 15 mg/kg orally in water (patient No. 1—carcinoma of the colon).

under the C × T curve for the oral doses and the "apparent availability" varied considerably between these two patients. Table 3 summarizes these data with the "availability" calculated by expressing the ratio of the area under the C × T (μg ml^{-1}min) curve of the oral dose and the area under the C × T curve of the iv dose as a percentage.

Bone Marrow Studies

The studies measuring the inhibition of uptake of radioactive thymidine and deoxyuridine as a function of plasma 5-FU concentration were performed on two patients receiving the drug iv and four patients receiving it orally. Although significant interpatient variation occurred, the initial depression of bone marrow cell uptake was much more rapid when the drug was administered iv. In addition, there was a more rapid occurrence of the nadir and subsequent recovery of marrow incorporation activity associated with the iv dose than with the oral dose. Figures 5 and 6 illustrate this uptake pattern for an oral and iv dose, respectively, in two different patients.

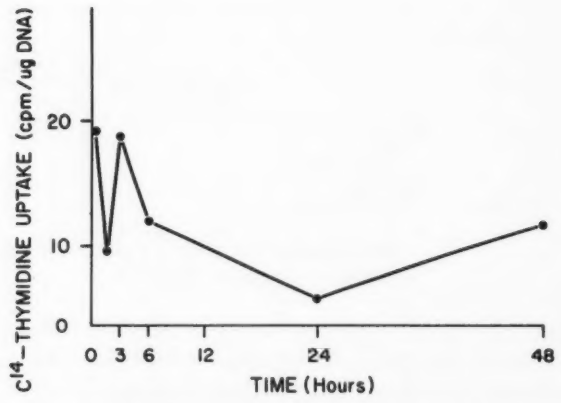

FIGURE 5.—Uptake of C^{14}-deoxyuridine into the bone marrow cells of a patient who received a single 15-mg/kg dose of 5-FU orally (patient No. 6—carcinoma of the colon metastatic to the liver).

The data for these six patients are summarized in table 4 and indicate (at least in this limited number of patients) that the bone marrow uptake pattern in the patients who received 5-FU orally and achieved "high" blood levels corresponds more closely with that of a patient receiving 5-FU iv than with a patient given 5-FU orally who evidenced low prolonged plasma levels.

In one of these patients enough bone marrow was withdrawn in each sample to allow a determination of the 5-FU concentration as well as the DNA uptake activity. This was thought desirable as a measure of reproducibility of the analytical method in a single patient since the plasma volumes withdrawn at any one time do not allow multiple gas chromatographic analysis of the same sample. These bone marrow fluid levels were relatively similar to plasma levels and are illustrated in table 5.

DISCUSSION

These studies were performed in conjunction with the large WCSG protocol 106 that compared the clinical response of randomized iv-oral 5-FU therapy in disseminated adenocarcinoma in an attempt to determine relationships between route of administration and the time-course of drug disposition. The results indicate significant differences in the plasma time-

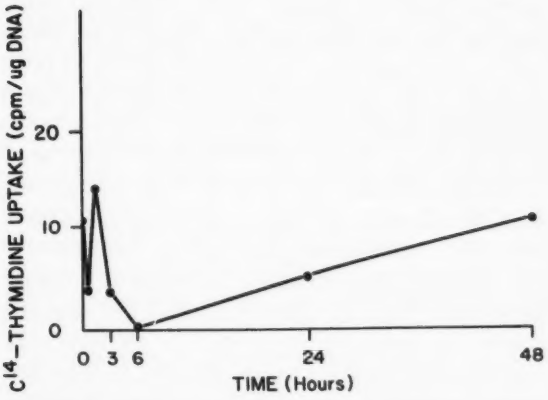

FIGURE 6.—Uptake of C^{14}-deoxyuridine into the bone marrow cells of a patient who received a single 15-mg/kg dose of 5-FU iv (patient No. 7—carcinoma of the pancreas).

course of free 5-FU after oral and iv administration which do not appear to reflect the differences in clinical response associated with these two routes of administration (10). Although the number of patients in a specific disease category was not sufficient to allow a correlation between plasma level and response, the plasma C × T data for two of the most commonly employed modes of 5-FU treatment were collected from a relatively large number of patients.

The pharmacokinetic data after a single iv dose of 5-FU were remarkably consistent in a heterogenous patient population and were in good agreement with data previously obtained by Mukherjee et al (14) and Clarkson et al (15).

TABLE 5.—Comparative 5-FU concentrations in plasma and bone marrow in a patient (No. 4) after oral administration

Time (min)	Plasma 5-FU concentration (μg/ml)	Bone marrow 5-FU concentration* (μg/ml)
0	–	–
15	1.08	+
30	9.30	11.2
45	11.40	+
60	2.85	+
90	2.05	+
120	1.15	+
180	0.65	1.30
240	~ 0.40	+
360	~ 0.38	~ 0.36
720	~ 0.42	+

* + = Samples were taken at 0 and 30 min and 3 and 6 hrs.

The disposition of the drug after oral administration was highly variable and less easily understood. In all oral doses given, measurable plasma levels were present for much longer times than in corresponding iv doses. Although slow and variable absorption (even from a solution) has been suggested previously (15), this does not completely explain these data. Rapid metabolism and the apparent importance of the first pass through the body would be expected to produce negligible plasma levels after the peak has occurred. The oral data are in agreement with a recently reported study by Douglass and Mittelman (17) which showed qualitatively similar peripheral concentrations of 5-FU following oral and iv administration of

TABLE 4.—Summary of bone marrow cell uptake of C^{14}-deoxyuridine

Patient No.	Route of administration	Peak plasma concentration of 5-FU (μg/ml)	Initial drop* (min)	Recovery† (hrs)
3	Oral	19.2	30	3
4	Oral	11.4	30	3
5	Oral	2.8	‡	‡
6	Oral	5.9	120	24
7	Iv	—	15	6
8	Iv	—	30	6

*Time of initial depression of C^{14}-deoxyuridine uptake.

†Time of initial recovery of bone marrow cell uptake activity.

‡Lowest activity occurred prior to drug administration; at all other times there was increased marrow activity.

5-FU-2-C^{14}. In addition, this study showed significant portal concentration and progressive biliary excretion in the case of an oral dose and a rapid decrease in biliary excretion after an iv dose. If this had been followed by reabsorption, the prolonged oral levels would become more understandable.

The oral data suggest a more significant first-pass effect and biliary excretion for patients who have liver malignancy, all of whom showed lower peak levels but longer residual levels. This lends support to the suggestion by Kung et al (9) that 5-FU might be more effective in patients with metastases to the liver due to high concentrations in the portal circulation. This cannot be demonstrated unequivocally until the relationship between portal or peripheral C × T and clinical response is documented.

The lower solubility of 5-FU in acidic medium (pKa 8.0 [18]) could account for the apparent decrease in absorption when the drug is administered in orange juice (pH 4) rather than in water or bicarbonate. This drug has an extremely slow rate of solubility so that precipitation in the small volume of acidic medium could result in less drug available for absorption even though the solubility of 5-FU in the gastric fluid may not be exceeded.

The term "availability" is used to compare total areas under the plasma C × T curves from various routes of administration. Because of the possible recycling of 5-FU, this availability is not synonymous with the amount of drug absorbed from an oral dose, but rather reflects the amount of drug in the plasma over time (C × T). The importance of this C × T relationship for cytotoxic drugs has been outlined by DeVita (19), and Jusko has developed quantitative pharmacodynamic models for both phase-specific (20) and phase-nonspecific (21) chemotherapeutic agents.

Our results, as well as the very recent data of Bruckner and Creasey (22) in a smaller population, support the need for more specific studies of C × T relationships for 5-FU in specific types of malignancies. A quantitative measure of clinical response as a function of 5-FU C × T data and the relationship of toxicity to 5-FU C × T data could be combined in a suitable model to predict more appropriate doses and schedules for 5-FU. Depression of bone marrow activity appears to be related to early plasma concentration after a single dose. Since 5-FU is thought to exert its cytotoxic activity by being converted to 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP) intracellularly, the detailed study of the distribution time-course of 5-FU and FdUMP may provide more significant C × T data than do the plasma concentrations of 5-FU. Methodology to improve the sensitivity of analytical methods is currently being developed. Further clinical studies are contemplated to compare oral and iv therapy for specific malignancies in an attempt to take advantage of the apparent differences on distribution due to the significant first-pass effect of this drug in the body.

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Failure of Indium 111-Labeled Bleomycin Tumor Scanning to Predict Response to Bleomycin (NSC-125066) Treatment [1,2,3]

Stephen E. Jones, Sydney E. Salmon, and Brian G. M. Durie [4,5]

Tumor scanning has become a useful tool in the clinical staging and followup of patients with cancer. Although various radionuclides such as Gallium 67 citrate are currently used for tumor scanning, recent efforts have been directed toward the labeling of drugs or tumor-specific antibodies that might selectively concentrate in tumors. One drug that has been of interest in this respect is bleomycin. A mixture of polypeptide antibiotics isolated from the fermentation products of *Streptomyces verticillus* (1), bleomycin is a new antineoplastic agent that has been shown to be clinically active, especially against squamous cell carcinomas, lymphomas, and testicular tumors (2). Moreover, studies by Umezawa et al (3) and Fujita et al (4) in animals have indicated that bleomycin concentrates specifically in skin, lung, and certain tumors. Because of the chemical propensity of bleomycin to form chelates with many bivalent and trivalent metallic ions, it has been possible to label the drug effectively for tumor scanning. So far, Indium 111 (^{111}In)-labeled bleomycin, which has favorable imaging characteristics, has received the most attention.

During our evaluation of ^{111}In-labeled bleomycin as a general purpose tumor scanning agent (5–7), we wondered whether or not those tumors that clearly accumulated radioactivity were the same tumors that might respond to treatment with therapeutic doses of bleomycin. That is, was bleomycin being taken up by tumor cells? Would a positive tumor scan predict responsiveness to treatment with bleomycin?

MATERIALS AND METHODS

Fourteen patients in whom tumor uptake of ^{111}In-labeled bleomycin had been demonstrated by tumor scanning were selected for study. All patients had advanced, measurable neoplasms for which there were strong clinical indications for a trial of therapy with bleomycin. Patients with squamous cell carcinoma were preferentially chosen for study and, of the 14 neoplasms, seven were squamous cell tumors (carcinoma of the head and neck in four patients, carcinoma of the uterine cervix in two patients, and carcinoma of the lung in one patient). The remaining tumors were ovarian adenocarcinoma (two patients), renal cell carcinoma (two patients), lung adenocarcinoma (one patient), fibrosarcoma (one patient), and diffuse lymphocytic lymphoma (one patient). Eight patients had not received prior chemotherapy; the other patients were refractory to standard treatment. Informed written consent was obtained prior to the start of treatment.

Tumor scans were performed in a standard fashion 48 hours after the intravenous administration of 3–5 millicuries of ^{111}In-labeled bleomycin (usual bleomycin dose, approximately 0.5 mg) (5–7). Therapeutic doses of bleomycin were administered intramuscularly twice weekly (dose level, 15 mg [units]). To be evaluable, patients had to receive at least 4 weeks of treatment with bleomycin.

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[2] Supported in part by Public Health Service grant CA-13612 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3] ^{111}In-labeled bleomycin (Tumor Scintigraphin) was generously supplied by Medi-Physics, Inc., Emeryville, Calif. Bleomycin was supplied by Bristol Laboratories, Syracuse, NY, through Cancer Therapy Evaluation, Division of Cancer Treatment, National Cancer Institute.

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RESULTS

There were no adverse reactions to tumor scanning with ^{111}In-labeled bleomycin. Thirteen of the 14 patients received bleomycin treatment for 4 weeks or more and were considered evaluable. The 14th patient (who had squamous cell carcinoma of the base of the tongue) experienced tumor progression during the first 2 weeks of bleomycin treatment and, consequently, the treatment was stopped. Four patients experienced fever or chills with treatment. No pulmonary toxicity was noted.

Only two of the 13 evaluable patients (15%) had objective evidence of tumor regressions of more than 50%. One of the responding patients had lymphoma and the other had squamous cell carcinoma of the cervix. These responses lasted only 2 and 3 months respectively. The other 11 patients had stable or slowly progressive tumor during the initial 4-week course of treatment and treatment with bleomycin was discontinued after this course.

DISCUSSION

When bleomycin is administered to laboratory animals, it is observed in high concentration in skin, lung, and certain tumors (3,4). In fact, Ichikawa et al (8) observed that in mice bleomycin treatment prevented the induction of squamous cell skin cancers but not sarcomas, and that the squamous cell tumors manifested significantly higher bleomycin uptake (as measured by bioassay and by radioactivity after administration of tritiated bleomycin) than did the sarcomas. In their system, tumor uptake of bleomycin correlated with response to treatment with bleomycin.

After the administration of ^{111}In-labeled bleomycin in man, radioactivity appears in 89% of a wide variety of solid tumors (7). Despite this high rate of success for imaging tumors, the results of this study indicate that in patients with positive tumor scans the rate of response to treatment with bleomycin is no greater than what would be expected from an otherwise unselected group of comparable patients (2). This lack of predictive value with ^{111}In-bleomycin scanning probably reflects the fact that the exact mechanism by which tumor labeling occurs is still unknown. It is not understood whether Indium 111 (dissociated from bleomycin), ^{111}In-labeled bleomycin, or some other as yet unidentified labeled intermediary compound is responsible for the appearance of radioactivity in tumor. Direct comparisons of the efficacy of tumor imaging with Indium 111 chloride versus ^{111}In-labeled bleomycin in man are not available, but the presence of bleomycin does appear to improve the frequency of tumor labeling since Indium 111 has only occasionally been observed in tumor tissue (9). Despite the lack of firm data on the mechanism of tumor labeling, there is at least circumstantial evidence that bleomycin is localized in tumor tissue when a positive image is seen because both tritiated bleomycin and bleomycin antibiotic activity have been found to selectively concentrate in animal tumors (3,4). From the biologic standpoint, these observations suggest to us that at least two essential (and perhaps separable) steps are necessary for bleomycin to exert its oncolytic effect: (*a*) selective drug uptake and (*b*) inhibition of DNA synthesis and cell death. Viewed in this context, bleomycin might have therapeutic potentials beyond those that can be currently achieved. Corollaries of this hypothesis are (*a*) that tumor localization of bleomycin could potentially be exploited as a component of combination chemotherapy (where bleomycin might be inactive as a single agent) and (*b*) that bleomycin could potentially serve as a "vehicle" for delivering intense local radioactivity or another cytotoxic moiety to the target tumor tissue.

The real test of the value of labeled bleomycin as a predictor of response will be possible when a tightly labeled bleomycin with fully preserved biologic activity is synthesized. Although the concept of attempting to predict future drug response with labeled anticancer drugs is not valid for ^{111}In-labeled bleomycin (as shown in this study), it may still prove to be of considerable value with other radiopharmaceuticals.

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Evaluation of Intravenous Allopurinol (NSC-1390) in Pediatric Neoplasia [1,2,3]

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Brief Reports and Preliminary Communications

Hyperuricemia and uric acid nephropathy are frequently associated with the dynamics of neoplastic growth because uric acid is one of the metabolic byproducts of cell degradation. This problem is intensified by current methods of chemotherapy and radiation therapy which rely largely on cell destruction for their efficacy (1).

Allopurinol, a structural isomer of hypoxanthine, was demonstrated to be capable of inhibiting xanthine oxidase by Elion et al in 1963 (2). Since that time, its clinical efficacy has been well documented (3–8) and it has been widely used as supportive therapy concomitant to pediatric antineoplastic therapy (9).

The catabolism of purine ribonucleotides in man yields the free purine bases, adenine, guanine, xanthine, and hypoxanthine. Of these bases, guanine is deaminated to xanthine by means of the enzyme xanthine oxidase. Xanthine oxidase also catalyzes the conversion of xanthine to uric acid, the end product of purine metabolism. Allopurinol has a 15-fold greater affinity for xanthine oxidase than the natural purine base xanthine; [7] it is therefore able to compete successfully for this enzyme and effectively inhibit uric acid production by blocking the conversion of hypoxanthine to xanthine and xanthine to uric acid. This inhibition occurs in the stages immediately preceding uric acid synthesis and in no way disrupts normal purine biosynthesis.

PATIENTS AND METHODS

Twenty-eight boys and 15 girls who were patients from The Children's Hospital Oncology Center, Denver, Colorado, were studied. The group included 27 patients with acute lymphocytic leukemia, two with lymphosarcoma, six with acute and one with chronic myelocytic leukemia, three with neuroblastoma, two with hepatoma, and two with Hodgkin's disease. All patients were selected for treatment or prophylaxis of hyperuricemia secondary to antineoplastic therapy according to the treatment protocol designed by The Children's Hospital Oncology Center.[8] These patients were either in the induction phase of therapy or in relapse and fell into one of the following categories: (*a*) newborn or young infants unable to take tablets; (*b*) postoperative tumor patients unable to take oral medication; (*c*) patients with severe vomiting; and (*d*) critically ill patients in need of intravenous (iv) therapy with fluids for infection or dehydration.

Allopurinol was administered iv in a dosage of 100–200 mg/m²/day divided in four doses every 6 hours. Doses were diluted in 25–50 cc of dextrose and water and were infused over a period of 15–20 minutes. The duration of iv allopurinol therapy ranged from 1 to 13 days based on the clinical indications and the need for iv therapy. Oral allopurinol therapy was reinstituted in patients as their conditions permitted. When iv allopurinol therapy was given,

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[2] Supported by Public Health Service grant CA-12247 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3] Allopurinol: CAS reg. No. 315-30-0; 1*H*-pyrazolo-[3,4-*d*]pyrimidin-4-ol. Supplied by the Burroughs Wellcome Co., Research Triangle Park, NC.

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[7] Burroughs Wellcome Co. A new concept in the therapy of hyperuricemia associated with gout and other conditions, 1966.

[8] Holton CP, and Mayer CM. Protocol for use of intravenous allopurinol (NSC-1390) in childhood neoplastic disease. The Children's Hospital, Denver, Colo, 1970.

iv fluids were calculated for maintenance of normal fluid and electrolyte levels. No attempt was made to overhydrate or alkalinize patients via iv infusions.

Serum uric acid levels were taken periodically by the uricase method to determine the therapeutic effect of iv allopurinol, and complete blood cell counts were taken to determine disease progression and response to antineoplastic therapy. A decrease in the white blood cell count in patients with leukemia in relapse or a regression in tumor mass in patients with solid tumors was interpreted as evidence for the efficacy of the chemotherapeutic agents employed. Also, if the serum uric acid level decreased or remained within normal limits (2.2–5.3 mg/100 ml in children under 15 years, 3.0–7.8 mg/100 ml in adult men) during an effective course of antineoplastic therapy, iv allopurinol therapy was considered effective.

TOXIC EFFECTS

Allopurinol (given iv) was well tolerated by all but one of the patients in this study. Among the reported side effects of allopurinol are gastrointestinal symptoms, marrow depression, fever, hepatomegaly, and skin rash (3,5,6,10). It should be noted that many patients in this study had these symptoms as a result of their disease or antineoplastic therapy. As a result, it was difficult in many cases to evaluate iv allopurinol for its toxic effects. It is of significance that no patient in this study evidenced fever, gastrointestinal symptoms, marrow depression, or hepatomegaly that could not be attributed to his disease or antineoplastic chemotherapy. In no case was it necessary to terminate iv allopurinol because of marrow depression.

The single incidence of allopurinol-associated toxicity occurred in a newly diagnosed 7-year-old boy who had acute lymphocytic leukemia. The patient had massive multiple organ involvement and leukemic heart disease with congestive heart failure. Iv allopurinol (25 mg four times a day) was administered daily concomitant to his induction medications which were given over a period of 14 days. On Day 15, iv allopurinol was discontinued and oral allopurinol was initiated (50 mg twice daily). The following day, a pruritic red maculopapular rash erupted around the extremities and trunk. Allopurinol was discontinued and the rash disappeared within 8 days.

RESULTS

A total of 43 patients were studied. One patient received two courses of iv allopurinol, and one patient received three courses. In 45 of the 46 drug courses analyzed, normal or near normal serum uric acid levels were attained. One patient, whose uric acid level remained elevated, received iv allopurinol therapy preceding death. Autopsy revealed tubular calcification, necrosis, and renal infection secondary to his leukemic process.

While receiving iv allopurinol therapy, eight patients (group 1) had partial or complete remission, 20 patients (group 2) had a partial response, and 18 patients (group 3) had no response to antineoplastic therapy. The data presented in table 1 demonstrate that iv allopurinol effectively lowered the serum uric acid levels in all patients studied, regardless of the uric acid level secondary to their disease or treatment.

The results of this study suggest that allopurinol can be safely administered to children

TABLE 1.—Serum uric acid levels of patients in each response group during iv allopurinol study

Group No.	No. of patients	Initial serum uric acid levels (mg/100 ml)		Nadir of serum uric acid levels (mg/100 ml)		Median decrease in uric acid levels (mg/100 ml)
		Range	Median	Range	Median	
1	8	4.0–12.8	6.0	1.8–4.6	3.1	2.9
2	20	2.0–11.0	5.8	1.2–5.2	3.3	2.5
3	18	1.8–18.0	6.0	0.0–9.8	3.7	2.3

iv without any severe toxic effects or loss of efficacy. If further testing bears out these results, iv allopurinol will represent a valuable means of combating hyperuricemia in children who are unable to tolerate oral medication.

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New Technique for the Measurement of Ascites Tumor Cell Numbers [1,2]

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Brief Reports and Preliminary Communications

In many studies on experimental neoplasms, a precise determination of the total tumor cell number is an important requisite. When ascitic tumors are used, such a measurement is generally performed by one of two types of procedures. The first procedure involves harvesting as much of the ascitic fluid as possible with repeated washings of the peritoneal cavity and counting the cells per unit volume. The second method requires weighing the tumor-bearing animal before and after fluid withdrawal and collection (1–4). These procedures have been used in the studies of Skipper and his group (5) on the growth kinetics of intraperitoneal (ip) leukemias and in early investigations of tumor allograft rejection (6,7). These methods are tedious, time-consuming, difficult to standardize, and of dubious precision, especially when the amount of ascitic fluid is low. The radioactivity dilution technique described in this paper appears to offer greater sensitivity and precision than the available methods while retaining the ease and economy of performance required for routine use.

Basically, the procedure consists of injecting ip in tumor-bearing hosts a standard volume of chromium 51 (^{51}Cr)-labeled erythrocytes. After mixing with the pre-existing fluid, measured aliquots of ascites are withdrawn, their radioactivity is determined, and the cell number per unit volume is counted. After correction for the volume injected, the total number of cells present in the cavity can be obtained by the formula: $\frac{\text{injected cpm}}{\text{withdrawn cpm}} \times$ cell concentration.

Sheep erythrocytes were labeled with ^{51}Cr as described by Perlmann et al (8), and a total volume of 0.6–1.0 ml, containing 3–6 × 10^4 cpm, was injected ip in the mice just after they were killed. Generally, two equal injections in the right and left side of the abdomen were used to facilitate a more even mixture of the injected cells with the ascites. After gentle massaging of the abdomen for 1 minute, the peritoneum was exposed and 0.2-ml aliquots of ascites were withdrawn from each side and mixed. The radioactivity of the sample was measured in a gamma spectrometer (Packard model 578) and the tumor cell number was determined microscopically. In our experience, the duration of massaging, at least in the 1–5-minute range, was not critical.

In order to comparatively evaluate this assay, normal CDF_1 mice were injected ip immediately after sacrifice with various numbers of L1210 leukemia cells suspended in a fixed volume of 2 ml of Eagle's medium. The total number of cells in the peritoneal cavity was determined in half of the animals by the radioactivity dilution technique described above; in the remaining mice cell number was estimated by syringe withdrawal of the fluid followed by three washings of the cavity with 2.5 ml of medium. In both cases the cell number per unit volume was counted microscopically. As shown in table 1, which gives the averaged results of two such experiments with six animals per dose, the total cell numbers determined by the radioactivity dilution technique were significantly nearer to the actual injected cell values than those estimated by the "washing" method. A curve drawn through the re-

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sults obtained by the dilution technique did not appear to be significantly different from that of the theoretical 100% cell estimate, at least over the range of cell numbers investigated. On the other hand, marked differences from the expected values were obtained when the washing technique was used, differences which ranged between –54% and +100%. It can also be seen that intragroup variation was markedly smaller with the radioactivity dilution technique. When a fixed number (5×10^7) of L1210 cells suspended in different volumes (0.5–3.0 ml) of medium was injected, a comparison of the two techniques again revealed that the radioactivity dilution method was superior to the washing technique (table 2).

In conclusion, the radioactivity dilution technique described appears to permit easier and more precise determination of the ascitic fluid volume, and hence of total cell numbers, than the method generally employed for this purpose. Since within 20 minutes after ip injection no significant radioactivity was detected in the portal and peripheral blood, indicating no substantial leakage of label from the peritoneal cavity, the assay could also be applied in living animals and has, in fact, been recently used with success in chemotherapy and tumor immunology studies. It is also conceivable that this approach could be exploited in other investigative fields where precise measurements of fluid volumes in body cavities are required.

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TABLE 1.—Comparison of total ascites cell numbers measured by the radioactivity dilution and the washing techniques after injection of varying inocula of L1210 leukemia in a constant volume*

No. of cells injected	No. of cells ($\times 10^6$) recovered			
	Radioactivity dilution technique	Difference compared to actual No. injected (%)	Washing technique	Difference compared to actual No. injected (%)
2.3	2.8 ± 0.6	+21	4.6 ± 3.1	+100
10	9.5 ± 0.32	–5	15.1 ± 3.8	+51
23	25.2 ± 1.25	+9	17.9 ± 4.3	–22.2
100	97.3 ± 5.44	–2.7	73.3 ± 16.1	–26.7
230	241.6 ± 11.76	+5	125.6 ± 48.9	–54.3

* Six mice per dose per experiment were used; data are means ± SE for 2 experiments. Cells were injected in a constant volume of 2 ml.

TABLE 2.—Comparison of total ascites cell numbers measured by the radioactivity dilution and the washing techniques after injection of a fixed cell number in varying volumes *

No. of cells injected	Volume injected (ml)	No. of cells ($\times 10^6$) recovered			
		Radioactivity dilution technique	Difference compared to actual No. injected (%)	Washing technique	Difference compared to actual No. injected (%)
5×10^7	0.5	46.9 ± 3.7	–6.4	33.2 ± 10.8	–34.6
	1.5	52.2 ± 4.0	+4.0	37.2 ± 12.5	–25.6
	3.0	51.5 ± 2.9	+3.0	42.6 ± 16.4	–12.8

* Six mice per dose per experiment were used; data are means ± SE for 2 experiments.

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Treatment of Experimental Mammary Adenocarcinoma With Herbicides [1,2]

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Brief Reports and Preliminary Communications

Many herbicides have selective effects on certain plant cells (1). The purpose of this investigation is to determine the effects of two herbicides, diphenamid (DPD) and picloram (PA),[5] on transplantable mammary adenocarcinoma (C3HBA) in mice.

MATERIALS AND METHODS

A mitosis inhibitor, DPD, and a protein synthesis inhibitor, PA, were dissolved in 95% ethanol and diluted with sterile water, and the pH was adjusted to 7.3. In experiment 1 each drug (10 mg/kg, 0.5 ml) was inoculated subcutaneously (sc) (lumbar area) into each respective experimental mouse. Three groups of adult male C3H mice consisting of 15 mice/group were used. The mice in all three groups were inoculated sc (inguinal area) with C3HBA tumor (50–60 mg; 12-gauge trocar). The mice were then inoculated daily with drug or saline: group I received DPD, group II received PA, and group III received 0.5 ml of saline. Every other day after the tumors became palpable, the greatest width and length measurements (longitudinal body axis) were taken with calipers. The tumor sizes in cm^3 were calculated according to the formula for an ellipsoid or a sphere:

$$V = \frac{\pi W^2 L}{6},$$

where W = width and L = length (2).

In experiment 2 the drugs were used in 20-mg/kg doses. Eighteen adult C3H mice (eight males and ten females) were used in each of three groups. The procedures were altered from experiment 1: the drugs were given in 0.5-ml sc injections daily for the first 12 days; beginning on the 14th day, drug injections were given on alternate days (0.25 ml, 10 mg/kg) until the mice died or the experiment ended. Control mice were given equivalent saline injections.

At death, tumors were cut into two parts. One part was placed in 12% formalin for histologic study; the other part was placed in 3% glutaraldehyde fixative for later electron microscopy studies.

Statistical differences between the mean tumor sizes were determined according to the "Student's t" test. Confidence limits were set at $P = 0.05$.

Thirteen groups of normal mice (five of each sex per group for 10- and 20-mg/kg doses; three of each sex for larger doses of 50–100 mg/kg of DPD or up to 40 mg/kg of PA) were treated for as long as 2 weeks to determine toxicity and LD50. Histologic data (all tissues) were obtained from mice receiving toxic and nontoxic levels of the drugs.

RESULTS

In experiment 1 the PA-treated group showed the least increase in tumor sizes (table 1). Survival times were as follows: control mice, 34.6 days (range, 4–56); DPD group, 28.3 days (range, 18–46); PA group, 31.2 days (range, 2–56). No significant differences were noted. In experiment 2 the PA- and DPD-treated tumors were consistently smaller than the control tumors (table 2). The control mice survived an average of 51.3 days (range, 36–81) and the DPD group survived an average of 44.1 days (range, 13–64) (P

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[5] Diphenamid: CAS reg. No. 957-51-7; *N,N*-dimethyl-2,2-diphenylacetamide. Supplied by Eli Lilly and Co.
Picloram: CAS reg. No. 1918-02-1; picolinic acid, 4-amino-3,5,6-trichloro-. Supplied by Dow Chemical Co.

TABLE 1.—Experiment 1: 10-mg/kg doses

Days posttransplant	DPD treatment: Mean tumor volume (cm^3) ± SD	DPD treatment: No. of mice	DPD treatment: *P* value*	Controls: Mean tumor volume (cm^3) ± SD	Controls: No. of mice	PA treatment: Mean tumor volume (cm^3) ± SD	PA treatment: No. of mice	PA treatment: *P* value*
8	0.09 ± 0.13	15	0.01†	0.28 ± 0.20	14	0.12 ± 0.13	13	0.05†
11	0.35 ± 0.28	15	0.10	0.71 ± 0.60	14	0.26 ± 0.24	13	0.025†
13	0.68 ± 0.57	15	0.025†	1.40 ± 0.90	13	0.50 ± 0.52	12	0.01†
15	1.19 ± 0.81	15	0.02†	2.39 ± 0.34	12	0.82 ± 0.95	12	0.001†
18	3.50 ± 1.57	14	0.70	4.00 ± 3.55	14	2.29 ± 2.22	13	0.20
20	4.30 ± 1.90	11	0.60	5.00 ± 4.10	14	3.40 ± 2.90	13	0.30
22	6.14 ± 3.10	11	0.90	5.85 ± 4.70	14	3.50 ± 3.30	11	0.20
25	8.50 ± 3.70	10	0.50	10.58 ± 8.70	13	4.90 ± 3.10	11	0.05†
27	14.36 ± 5.40	8	0.40	11.40 ± 7.80	11	7.70 ± 4.50	11	0.25
29	17.00 ± 4.70	6	0.60	14.71 ± 9.10	10	11.60 ± 5.40	9	0.40
32				18.16 ± 8.10	10	13.73 ± 7.60	7	0.30
34				19.19 ± 6.50	9	14.21 ± 9.60	5	0.40
36				25.67 ± 8.40	8	18.00 ± 10.60	5	0.25

*Statistical probabilities between each drug-treated group and the control group.
†Significant difference.

TABLE 2.—Experiment 2: 20-mg/kg doses

Days posttransplant	DPD treatment: Mean tumor volume (cm^3) ± SD	DPD treatment: No. of mice	DPD treatment: *P* value*	Controls: Mean tumor volume (cm^3) ± SD	Controls: No. of mice	PA treatment: Mean tumor volume (cm^3 ± SD)	PA treatment: No. of mice	PA treatment: *P* value*
8	0.04 ± 0.08	18	0.005†	0.20 ± 0.19	18	0.05 ± 0.01	18	0.01†
11	0.12 ± 0.09	17	0.02†	0.40 ± 0.43	18	0.18 ± 0.29	17	0.10
13	0.19 ± 0.13	17	0.05†	0.50 ± 0.59	18	0.18 ± 0.19	17	0.05†
15	0.50 ± 0.17	17	0.025†	1.08 ± 0.89	18	0.27 ± 0.11	17	0.005†
18	1.17 ± 1.40	17	0.90	1.15 ± 1.03	18	0.67 ± 0.45	17	0.10
20	1.63 ± 2.30	18	0.90	1.60 ± 1.38	18	1.25 ± 1.42	17	0.20
22	2.03 ± 2.97	16	0.80	2.33 ± 2.00	18	1.77 ± 2.35	17	0.50
25	2.95 ± 5.11	16	0.70	3.68 ± 4.11	18	2.40 ± 4.10	16	0.40
27	3.54 ± 5.30	16	0.50	4.79 ± 5.00	18	2.26 ± 5.91	15	0.25
29	4.88 ± 6.60	16	0.50	6.37 ± 6.10	18	3.94 ± 6.66	15	0.40
32	5.51 ± 6.23	16	0.20	9.44 ± 7.75	18	4.12 ± 7.15	14	0.10
34	8.06 ± 8.40	16	0.25	12.02 ± 9.10	18	2.25 ± 2.60	12	0.001†
36	7.85 ± 5.83	15	0.10	12.70 ± 8.30	17	3.98 ± 4.37	11	0.005†
39	13.00 ± 9.20	13	0.40	16.54 ± 8.49	15	4.31 ± 6.08	10	0.001†
41	12.10 ± 8.40	11	0.005†	25.00 ± 11.54	14	3.74 ± 4.35	10	0.001†
43	13.80 ± 9.30	11	0.01†	27.40 ± 12.06	14	6.05 ± 6.53	10	0.001†
46	13.50 ± 7.34	8	0.001†	39.20 ± 17.78	11	8.42 ± 8.35	10	0.001†
48	16.10 ± 7.45	8	0.001†	35.10 ± 11.82	11	6.44 ± 7.75	6	0.001†
50	16.70 ± 6.50	7	0.005†	31.30 ± 7.71	10	4.84 ± 6.75	5	0.001†
53	20.00 ± 7.47	5	0.01†	37.10 ± 10.10	8	5.97 ± 8.00	5	0.001†

*Statistical probabilities between each drug-treated group and the control group.
†Significant difference.

>0.10). Three of the 18 PA-treated mice (16.7%) were alive when drugs were stopped on the 82nd day. Two of the remaining mice no longer had tumors and appeared to be cured (11%). One of these mice (male) had a maximum tumor size of 0.91 cm^3 at 15 days (gone by the 29th day). This mouse died on the 91st day of an unknown cause (no tumor at death). The other mouse (female) had a tumor of 0.18 cm^3 at 15 days (gone by the 18th day). This mouse is alive 184 days after transplant. The one mouse that still had a tumor on the 82nd day (24.18 cm^3) died on the 89th day. Nine of the 18 PA-treated mice, including the three survivors, had marked, generalized edema at about the 14th day.

Normal mice became lethargic shortly after PA injection. At 10 mg/kg the mice became drowsy for 20–30 minutes. At 20 mg/kg the lethargy was more pronounced (1 hour). At 30 mg/kg the mice were lethargic for over 1 hour and three of six died on the fourth day (LD50 = 30 mg/kg). Apparently, PA is more toxic to animals with tumors since edema occurred only in tumorous mice. DPD at doses of 10 and 20 mg/kg in normal mice did not produce toxic effects. The LD50 is about 95 mg/kg with some mortality (LD10) occurring at 80 mg/kg. The mice became tremorous at 65 mg/kg and were comatose for over 1 hour at 70 mg/kg.

No histologic (7μ; hematoxylin and eosin) differences were noted in the experimental versus the control mice. The tumors of each group were about 50% necrotic. No toxic effects were noted in the tissues of normal mice receiving experimental dose levels of the drugs. Severe fatty metamorphosis was noted in the livers of mice receiving lethal dosages of both drugs.

The tumor sizes of experiment 2 mice did not differ according to sex. Weight losses for experiment 2 mice were 15% for PA, 7% for DPD, and 11% for controls.

DISCUSSION

According to the LD50 data, DPD may be used at higher dosage levels than PA. More experimentation is planned.

The 11% survival and the inhibition of tumor growth in the PA-treated mice are intriguing and suggest an urgency to investigate the chemotherapeutic potential of herbicides.

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Adriamycin (NSC-123127) Toxicity: Unusual Melanotic Reaction[1,2]

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Brief Reports and Preliminary Communications

Adriamycin is an anthracycline antibiotic isolated from cultures of *Streptomyces peucetius*. It has a wide spectrum of clinical antitumor activity with favorable results reported in lymphomas, breast and bladder cancers, tumors in children, and a variety of sarcomas. The major toxic reactions have been marrow depression, alopecia, stomatitis, and cardiomyopathy (1). O'Bryan et al reported skin pigmentation in two of 472 patients (2), but in general this reaction has not been prominent.

The purpose of this paper is to report an unusual melanotic pigmentary reaction to adriamycin therapy.

CASE REPORT

A 70-year-old black woman was referred for chemotherapy because of a transitional-cell carcinoma of the bladder. In 1956, she had carcinoma of the cervix (stage 1b) treated by insertion of radon seeds. In December 1967, the patient had epidermoid carcinoma of the vulva with metastases to the left inguinal nodes that was treated with 5000 rads of external radiotherapy (betatron). During the course of this therapy, radiation cystitis and severe hemorrhagic proctitis developed, the latter requiring a transfusion of 6 units of blood in December 1968, and 2 more units in February 1969. Thereafter, she did well for nearly 4 years. In May 1973, she returned to the hospital after 8 months of intermittent hematuria and dysuria. Examination revealed a slightly obese black woman most of whose skin color was a light- to medium-chocolate brown. There was brawny edema of both labia, and partial depigmentation of the labia in the previously irradiated area. An intravenous (iv) pyelogram was not diagnostic, but a cystoscopy revealed a solid tumor arising from a broad base on the dome of the bladder; a biopsy showed transitional-cell carcinoma.

The patient was given a total of 397 mg (205 mg/m^2) of adriamycin iv in three courses over a period of 6 weeks (60–80 mg/m^2 every 3 weeks). The first two courses were divided into three successive daily doses; the third course was given in a single infusion.

Alopecia began 10 days after adriamycin therapy was started and was nearly total at the end of 2 months. Stomatitis and vulvitis were present at 6–7 weeks. Leukopenia was at its nadir (white blood cell [wbc] count 1700 cells/mm^3) after 7 weeks, but thrombocytopenia was not observed.

None of these reactions were disturbing to the patient. However, 6 weeks after therapy was started the patient reported an increased pigmentation of the palms. The final dose of 148 mg of adriamycin was given on that date. Three weeks later, the patient's chief complaint was the occurrence of diffuse and uniform brown-black hyperpigmentation of the palms, soles, and proximal nailbeds (figs 1 and 2). Close inspection revealed that in addition to the prominent changes noted on the palms, soles, and nailbeds, the dorsa of the knuckles had also darkened and there were several small hyperpigmented areas on the buccal mucosa. A biopsy of hyperpigmented skin from the dorsum of a knuckle showed increased melanin deposition with an increased number of melanocytes in the basal layer of the epidermis.

The patient was most distressed by her appearance despite the reassurances of her family who sought to minimize the importance of the

[1] Received Feb 27, 1974; accepted Apr 18, 1974.

[2] Adriamycin was supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, National Cancer Institute.

[3] Princeton Medical Center, NJ.

[4] *Reprint requests to:* Dr. Harvey Rothberg, Princeton Medical Group, Medical Arts Building—Suite B, Witherspoon and Franklin Sts, Princeton, NJ 08540.

FIGURE 1

FIGURE 2

pigmentary changes. She was quite embarrassed, was reluctant to appear in stores and other public places, and insisted on wearing gloves when she was in church.

Most of the patient's major symptoms of lower abdominal pain and hematuria had disappeared and she was vigorously reassured. When seen 5 weeks later, lightening of the previously darkened skin was evident. By the end of 2 more months, her palms, soles, and buccal mucosa had returned to normal appearance and the dark melanotic area on the fingernails occupied a broad horizontal band on roughly the middle third of each nail, apparently migrating outward with the progressive growth of the nail. Three months later, abdominal pain and dysuria recurred, but the patient was reluctant to consider further chemotherapy. She gradually weakened and finally died 11 months after the start of adriamycin therapy.

COMMENTS

Hypermelanosis in man is generally due to an increase in the production of melanosomes and their subsequent distribution to the keratinocytes (3). This may be induced by polypeptide hormones (melanocyte-stimulating hormone and adrenocorticotropic hormone, as in Addison's disease or pituitary tumors) or by drugs (such as psoralens or busulfan [5]). The pathogenesis of the hypermelanosis in this patient is obscure, but there was no apparent endocrinopathy, and no other drugs were given. The time relationship of the pigmentary

[5] Busulfan: NSC-750; CAS reg. No. 55-98-1; 1,4-butanediol, dimethanesulfonate.

changes to the administration of adriamycin in this patient clearly implicates adriamycin as the causative agent. The clinical effects were striking and most distressing to the patient. Therefore, though it may be uncommon, it would appear that hypermelanosis should be added to the list of possible toxic effects of adriamycin. Whether this effect has a racial predilection remains uncertain.

ADDENDUM

Subsequent to acceptance of this manuscript, another report describing hyperpigmentation of nails and palmar creases in ten of 52 children treated with adriamycin has been published (4). In this series, the pigmentary changes were more common and more prominent in black children than in white children.

REFERENCES

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Central Nervous System Complications After Combination Treatment With Adriamycin (NSC-123127) and 5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388)[1,2]

Brief Reports and Preliminary Communications

Richard A. Gams and John T. Carpenter[3,4]

Gerner et al (1) recently described their experience in the treatment of disseminated melanoma with 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) and actinomycin D. During the course of the study cerebral hemorrhage suddenly developed in five patients despite the fact that previous studies had not revealed cerebral metastases. The complication occurred 3–4 weeks after the last course of therapy. The patients usually had headache or a stroke-like clinical status followed by rapid deterioration and death. In four of the five patients cerebral hemorrhage was confirmed by postmortem examination, and the authors speculated that the tumoricidal effect of the agents on cerebral metastases resulted in tumor necrosis and secondary hemorrhage. They suspected that NSC-45388 was the responsible agent and presented evidence that it crosses the blood-brain barrier. The authors further stated that they could find no reference to this complication in the literature.

We have recently been evaluating the combination of adriamycin (NSC-123127) and NSC-45388 in a variety of solid tumors (University of Alabama Project No. 204; Chairman, Dr. George Omura, University of Alabama Medical Center). During the course of these studies two patients with malignant melanoma and one with a soft-part sarcoma had seizures after treatment with this combination of agents.

CASE REPORTS

Patient No. 1.—Patient No. 1 was a 32-year-old white woman who first noted a mass in the left groin in February 1973. Because of a progression in the growth of this mass and the appearance of additional masses, a biopsy was performed in August 1973 which demonstrated malignant melanoma. Because of the existence of a large left obturator node, the patient was felt to be inoperable. The results of neurologic examination and initial laboratory studies were normal. Consequently, the patient was treated with adriamycin (60 mg/m²) on Day 1 and NSC-45388 (625 mg/m²) on Days 1 and 4, the course being repeated every 3 weeks. She received courses of this combination on September 4, September 25, and October 16. At the beginning of the third course of therapy she complained of episodic twitching of the left arm. She also stated that she had several episodes of transient paralysis of that arm, as well as difficulty in balance. She denied generalized seizure activity. On October 19, a brain scan was done and showed a lesion in the left parietal-parasagittal region. She was given Dilantin (100 mg three times daily) and received a course of total cranial irradiation. She continued to experience focal seizure activity and trouble with equilibrium, although these were decreased in severity. There was no significant change in the size of her lesions and the therapeutic trial was discontinued.

Patient No. 2.—Patient No. 2 was a 50-year-old white man who underwent resection of a malignant melanoma from his left chest in July 1970. He did well until 1972 when nodules reappeared in the area of previous surgery. A wide resection and left mastectomy were performed. He was again asymptomatic until April 1973, when nodules on the left chest were again found and removed. In all instances the biopsies revealed malignant melanoma. He remained free of obvious disease until August

[1] Received Apr 16, 1974; accepted Apr 19, 1974.

[2] NSC-45388: CAS reg. No. 4342-03-4; DTIC.

[3] University of Alabama Medical Center, Birmingham.

[4] *Reprint requests to:* Richard A. Gams, MD, The University of Alabama Medical Center, University Station, Birmingham, Ala 35294.

1973, at which time a lesion in the right lower lobe was discovered from a chest roentgenogram. He received courses of adriamycin-NSC-45388 in the doses reported for patient No. 1. These courses were given on August 10, September 21, October 12, November 2, and November 26. The lesion in the right lower lobe which measured 4 × 4 cm at the initial visit decreased to a minimum size of 0.5 × 0.5 cm. On the night of August 31, the date of the second course of therapy, the patient had generalized grand mal seizures. Lumbar puncture, brain scan, and electroencephalogram failed to reveal evidence of central nervous system metastases. However, he was given a course of cranial irradiation and began receiving Dilantin (100 mg four times a day) and phenobarbital (32 mg four times a day). His seizures were eventually controlled by this combination of anticonvulsants. Two weeks after his last course of therapy the patient suddenly died at home. No autopsy was obtained.

Patient No. 3.—Patient No. 3 was a 64-year-old white man who was found to have a mass in the left sternoclavicular area in May 1973. Operation revealed a mass attached to the periosteum of the clavicle and the second rib. Microscopic evaluation revealed a pleomorphic soft-part sarcoma. A repeat chest roentgenogram revealed multiple pulmonary metastases. Consequently, the patient was given a course of adriamycin-NSC-45388 in the doses reported for patient No. 1. These courses were given on May 25 and June 22, 1973. On the night of June 22, 1973, focal seizures beginning in the right arm and progressing to grand mal seizures developed. Brain scan revealed lesions in the area of the left central sulcus and below the right postcentral gyrus. The patient's seizures were controlled with Dilantin (100 mg three times a day). Nevertheless, over the next 2 weeks there was progressive deterioration in his condition and he died at home. No autopsy was obtained.

Although some patients with known central nervous system metastases have improved after treatment with NSC-45388 (1), serious adverse effects possibly related to treatment should be anticipated. Every patient given this medication should undergo careful neurologic evaluation perhaps including brain scan in an attempt to anticipate central nervous system complications.

REFERENCE

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ANNOUNCEMENTS

Symposium on "Late Consequences of Successful Cancer Therapy: Proven and Potential"

Orlando, Florida, January 9–11, 1975
Sheraton Olympic Villas

This symposium is sponsored by the Clinical Cancer Investigation Review Committee of the National Cancer Institute. The Program Chairman is Giulio J. D'Angio, MD, Memorial Sloan-Kettering Cancer Center. It is open to physicians interested in cancer therapy; no registration fee will be charged.

Course in Cancer Chemotherapy

The Memorial Sloan-Kettering Cancer Center will present a 1-week course in cancer chemotherapy to be held December 2–6, 1974.

This course is designed principally for physicians interested in medical oncology and clinical investigations in cancer chemotherapy. It will include lectures and panels on screening methods, pharmacologic techniques, and methods for clinical evaluation of potential chemotherapeutic agents. The chemistry and pharmacology of agents used in the treatment of cancer will be reviewed. There will be a strong emphasis on disease-oriented "workshops" which will consider optimal methods of patient management relating medical oncology to other relevant disciplines.

Applications should be submitted as soon as possible as enrollment is limited. The fee of $150 is payable at the time of application. Requests for registration forms and details of the program should be addressed to: Irwin H. Krakoff, MD, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

Fellowship in Medical Oncology

Fellowships are available in Medical Oncology at the University of Mississippi Medical Center in Jackson, Mississippi. The program is for two years and involves participation in cooperative treatment programs as part of the Southwest Oncology Group. Opportunity will also be provided to participate in the active Hematology Service as well as Radiation Therapy and Pathology. Two years of medical residency are required as a minimum. The stipend is adjusted relative to experience.

Inquiries should be directed to Dr. Francis S. Morrison, Associate Professor of Medicine, Chief, Division of Hematology and Oncology, University of Mississippi Medical Center, Jackson, Miss 39216.

Second National Hellenic Congress of Oncology

Athens, Greece, April 20–23, 1975

The Second National Hellenic Congress of Oncology will be held in Athens, Greece, on April 20–23, 1975. The following topics will be covered: (1) cancer of the lung, (2) cancer of the colon, (3) primary tumors of the bones, (4) tumors of the male genitalia, (5) virus-induced tumors in humans, and (6) experimental chemotherapy-immunotherapy of neoplasms. Panel discussions will include: (1) organization for cancer control, (2) charlatanism and cancer, and (3) enthanasia and cancer.

The official language of the Congress will be Greek with English and French as additional languages. The time provided for each short presentation on the main topic and symposia will be limited to 8 minutes including slide projections. The titles of the papers must be submitted prior to September 15, 1974, and the summaries, not exceeding 150 words in each language, in English and French, prior to November 30, 1974. In order to facilitate the publication of the proceedings, the full text should be submitted to the Secretariat of the Congress not later than March 1, 1975. The full text should not exceed 5 pages, typed double spaced, including figures and tables. Films on topics of the Congress will be projected and should be in 16-mm format and limited to 10 minutes.

Registration fees: Members Drs 900; assistant physicians Drs 400, technicians, social assistants, and nurses not presenting a paper Drs 200, if sent to the Secretariat before September 15, 1974. After this date, the amount of fees will be doubled, except for the last category. It should be noted that no paper will be accepted for presentation unless it is submitted together with the registration fees of all authors.

Visits to scientific institutions, museums, and archaeological sites will be organized.

For general information contact: Secretariat of the Second National Hellenic Congress of Oncology, Congress Organization Centre, Loukianou 25, Athens 139, Greece (Tel 719615).

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